Title: IL-2 is an upstream regulator of CD4$^+$ T cells from visceral leishmaniasis patients with therapeutic potential.

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Summary: Transcriptional changes in CD4$^+$ T cells during visceral leishmaniasis caused by *Leishmania donovani* infection were examined and IL-2 was identified as an important upstream regulator of these cells. Functional studies demonstrated the therapeutic potential of IL-2 for improving anti-parasitic immunity.

Footnotes

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Abstract

Control of visceral leishmaniasis (VL) caused by *Leishmania donovani* requires IFNγ production by $CD4^+$ T cells. In VL patients, anti-parasitic $CD4^+$ T cell responses are ineffective for unknown reasons. In this study, we measured the expression of genes associated with various immune functions in these cells from VL patients and compared them to $CD4^+$ T cells from the same patients after drug treatment and from endemic controls. We found reduced *GATA3*, *RORC* and *FOXP3* gene expression in VL patient $CD4^+$ T cells, associated with reduced Th2, Th17 and $FOXP3^+$ $CD4^+$ T regulatory cell frequencies in VL patient blood. IL-2 was an important upstream regulator of $CD4^+$ T cells from VL patients, and functional studies demonstrated the therapeutic potential of IL-2 for improving anti-parasitic immunity. Together, these results provide new insights into the characteristics of $CD4^+$ T cells from VL patients that can be used to improve anti-parasitic immune responses.

Key Words: Visceral leishmaniasis, *Leishmania donovani*, $CD4^+$ T cells, IL-2

Introduction

$CD4^+$ T cells play key roles in immunity against many different pathogens [1]. Intracellular protozoan parasites like *Leishmania donovani* are controlled by $IFN\gamma^+$ $Tbet^+$ $CD4^+$ T (Th1) cells [2]. The pro-inflammatory cytokines produced by Th1 cells help phagocytes kill captured or resident parasites by directly stimulating anti-microbial pathways [3, 4]. However, if infection persists, a juxtaposing pattern of inflammation and immune regulation can be established, resulting in $CD4^+$ T cell dysfunction and associated disease [5, 6].

Interleukin 2 (IL-2) is an important cytokine that influences T cell behaviour [7]. It is required for the survival, proliferation and differentiation of $CD4^+$ T cells, $CD8^+$ T cells and NK cells [8], by binding to either the high affinity trimeric IL-2 receptor (IL-2R) made up of IL-2Rα (CD25), IL-2Rβ (CD122) and IL-2Rγ (CD132) or the dimeric IL-2R (comprising β and γ chains) [9]. The trimeric IL-2R receptor is highly expressed on activated $CD4^+$ T cells and $Foxp3^+$ $CD4^+$ T regulatory (Treg) cells, while memory $CD8^+$ T cells and NK cells express high levels of the dimeric IL-2R [7].

L. donovani-infected mice receiving IL-2 blocking mAb failed to control hepatic parasite growth, associated with impaired granuloma development, while infected mice treated with exogenous IL-2 had reduced liver parasite burdens and increased granuloma development, relative to controls [10]. Similarly, intra-nodular injection of recombinant IL-2 in patients with disseminated cutaneous leishmaniasis reduced parasite numbers, associated with $CD4^+$ T cell infiltration [11]. However, the therapeutic application of IL-2 has had limitations because of its short half-life [12] and adverse side effects [13].

Combining recombinant IL-2 with certain IL-2-reactive monoclonal antibodies (mAbs) can preserve IL-2 signalling capacity and enhance cytokine half-life *in-vivo* [14]. Additionally different IL-2 mAbs expose different IL-2R binding sites when bound to recombinant IL-2 [15]. For example, injecting IL-2 conjugated to the JES6.1A12 anti-IL-2 mAb (IL-2Jc) into mice led to selective stimulation and expansion of $CD25^+$ T cells, but not $CD25^-$ T cells [15].

In this study, we measured the expression of a defined set of genes in peripheral blood $CD4^+$ T cells from visceral leishmaniasis (VL) patients infected with *L. donovani* on presentation to clinic and 30 days after drug treatment, as well as in the same cells from endemic controls (ECs). We identified IL-2 as a major upstream signalling molecule in $CD4^+$ T cells from VL

patients with active disease, and examined whether this cytokine signalling pathway could be manipulated for therapeutic advantage.

Materials and Methods

Sample collection

All patients presented with symptoms of VL at Kala-Azar Medical Research Centre, Muzaffarpur, Bihar, India for diagnosis and treatment. Diagnosis was performed by detection of anti-rK39 antibodies in the serum and/or amastigotes in splenic biopsies. Patients were treated with a single dose of Ambisome (10mg/kg) administered intravenously. Blood was collected on admission to hospital and 30 days after drug treatment. The study was approved by the Institute of Medical Sciences, Banaras Hindu University Ethics Committee and all subjects provided written informed consent. Clinical data for all subjects enrolled in the study is presented in Supplementary Table 1. Heparinized venous blood was collected from patients (n=82) and endemic healthy subjects (n=68). All patients were HIV negative and above twelve years of age.

Isolation of $CD4^+$ T cell RNA, quality control and gene expression analysis

$CD4^+$ T cells were isolated from peripheral blood mononuclear cells (PBMC), RNA isolated, quality controlled and subjected to gene expression analysis on the NanoString gene expression platform (NanoString Technologies, Seattle, WA) using a code set consisting of a gene panel related to T cell biology, activation, differentiation and regulation, as previously described [16] (Supplementary Table 2). Gene expression data was normalized for each sample prior to differential gene expression (DGE) analysis [16]. Briefly, after QC assessment and background subtraction, count values were normalised using the geometric mean of housekeeping genes (*ACTB*, *B2M*, *GAPDH*, *HPRT1* and *RPLP0*). Comparisons between two groups were made using a paired *t*-test for day 0 and day 30 comparisons, or an unpaired *t*-test for day 0 or day 30 comparisons to ECs. *p* values were adjusted for multiple testing using the Benjamini-Hochberg method where the false discovery rate (*q*-value; p-value false discovery rate (FDR)) considered significant was $q \leq 0.05$.

Real Time PCR

Real-time qPCR was performed using TaqMan based chemistries with FAM MGB-labelled primer/probes to measure mRNA expression, while VIC-MGB labelled 18S rRNA was used as an endogenous control, as previously described [17].

Antibodies

Fluorescently conjugated antibodies against CD4 (RPA-T4), CD3ε (UCHT1), FOXP3 (236A/E7), GATA3 (L50-823), RORγt (Q21-559), TBET (4B10), CTLA4 (BNI3), CD96 (6F9), CCR6 (11A9), CCR4 (1G1), CD38 (HIT2), CD40L (89-76) and IFNγR1 (GIR-208) (BD Biosciences, San Diego, CA), as well as Aqua Zombie viable dye (Biolegend, San Diego, CA), were used for flow cytometry studies on human samples.

FACS analysis of $CD4^+$ T cells

PBMCs were stained for cell surface and intracellular markers as previously described. Cells were analyzed using CellQuest Pro (BD Biosciences) and FlowJo software (Trees tar Inc, Ashland, OR). Gates were set using fluorescence minus one (FMO) controls. Analysis was performed by gating on $CD3\varepsilon^+$ $CD4^+$ T cells and then measuring the molecule of interest.

Ex vivo whole blood assay

Recombinant human IL-2 (1μg/ml) (R & D Systems, Minneapolis, MN) or vehicle (PBS) was added to whole blood assays [17], as indicated in Figure legends. IFNγ and IL-10 levels in cell culture supernatants were measured by ELISA [18].

L. donovani infections of C57BL/6 mice

L. donovani (LV9; MHOM/ET/67/HU3) [19] amastigotes ($2x10^7$) were injected intravenously (i.v.) into experimental mice. Hepatic parasite burdens were expressed in Leishman-Donovan units (LDU), as previously described [20]. Splenic parasite burden was determined by limiting dilution assay [18].

Mice

Female C57BL/6 mice were purchased from the Animal Resource Centre (ARC; Canning Vale, WA, Australia). B6.Foxp3.DTR [21] were bred and at QIMR Berghofer. Mice were age- and sex-matched and maintained under pathogen-free conditions. All procedures were conducted with approval of the QIMR Animal Ethics Committee (A02-634M), and in accordance with the "Australian Code of Practice for the Care and Use of Animals for Scientific Purposes" (NHMRC, Canberra, Australia).

Administration of IL-2/anti-IL-2 complexes to mice

Anti-mouse IL-2 (JES6-1A12) was purified from cell culture supernatant by protein G column chromatography (Amersham, Uppsala, Sweden), followed by endotoxin removal (Mustang Membranes, Pall Life Sciences, East Hills, NY). 1.5µg of recombinant murine IL-2 (eBioscience, San Diego, CA) was incubated with 50µg of JES6-1A12 mAb in saline for 30 minutes at 37°C, as previously reported [15], prior to intra-peritoneal (i.p.) administration in a volume of 200 µL on days 14 and 21 p.i..

$CD4^+$ T cell depletion in mice

$CD4^+$ T cells were depleted as described previously [22]. $Foxp3^+$ Treg cells in B6.Foxp3.DTR animals were depleted via i.p. administration of diphtheria toxin (DT) [23]. Efficiency of cell depletion was greater than 95% for $CD4^+$ T cells and Treg cells, as previously reported [22, 23].

Statistical analysis

Statistical analysis was performed using GraphPad Prism 6 (GraphPad Software, La Jolla, CA). Analysis of human cellular assays was performed using Wilcoxon matched-pairs signed rank test or non-parametric Mann-Whitney tests, as appropriate. Analysis of mouse data used Mann-Whitney tests for comparisons between two groups, and a One-Way ANOVA to assess more than 2 groups within an experiment. $p < 0.05$ was considered significant.

Results

A CD4$^+$ T cell gene signature from VL patients

Peripheral blood CD4$^+$ T cells were isolated from VL patients prior to anti-parasitic drug treatment (Day 0; VL D0), the same patients 30 days after commencement of drug treatment at hospital discharge (Day 30; VL DIS) and from healthy, endemic controls (ECs). RNA was isolated and targeted transcriptional profiling of 130 genes associated with T cell activation, differentiation, effector functions and immune regulation was performed, as previously described [16]. Interestingly, we found more transcriptionally down-regulated than up-regulated genes when we compared CD4$^+$ T cells from VL patients before drug treatment with time of discharge (Figure 1A), similar numbers of down- and up-regulated genes when compared with CD4$^+$ T cells from ECs (Figure 1B), and more transcriptionally up-regulated than down-regulated genes when we compared CD4$^+$ T cells from VL patients at time of discharge with ECs (Figure 1C; Supplementary Figure 1).

Genes encoding several important CD4$^+$ T cell lineage-defining transcription factors had decreased expression in CD4$^+$ T cells from VL patients, compared with cells from the same patients after drug treatment and from ECs. These differentially expressed genes (DEGs) included *GATA3*, *RORC* and *FOXP3*, associated with Th2, Th17 and regulatory T (Treg) cells, respectively. Consistent with transcription data, we found reduced frequencies of GATA3$^+$, RORCγ$^+$ (encoded by *RORC*) and FOXP3$^+$ CD4$^+$ T cells in the peripheral blood of VL patients, compared with CD4$^+$ T cells the same patients after drug treatment and from ECs, although the decrease in FOXP3$^+$ CD4$^+$ T cells did not always reach statistical significance (Figure 2A-B). Interestingly, despite no significant differences in CD4$^+$ T cell *TBX21* gene (encoding TBET) expression between groups, we found significantly higher frequencies of TBET$^+$ CD4$^+$ T cells in the peripheral blood of VL patients, compared to the same patients after drug treatment, but no difference compared to ECs (Figure 2A-B). These data show Indian VL patients have reduced peripheral blood Th2, Th17 and Treg cell frequencies, and a decreased Th1 cell frequency in VL patients following drug treatment.

Consistent with DEG data, we found an increased frequency of CD4$^+$ T cells from VL patients expressing the immunoregulatory molecules CD38 and CTLA4, compared to CD4$^+$ T cells from the same patients 30 days after drug treatment and ECs (Figure 3A-B). In contrast, but again consistent with DEG data, we found a decreased frequency of CD4$^+$ T cells from VL

patients expressing the chemokine receptors CCR4 and CCR6, as well as the important cell signalling molecules CD40LG, IFNGR1 and CD96, compared to control groups (Figure 3A-B). Thus, $CD4^+$ T cells from VL patients had DEGs encoding a broad range immunoregulatory and inflammatory molecules.

IL-2 is a major upstream regulator of $CD4^+$ T cells from VL patients

We next compared DEGs in $CD4^+$ T cells from VL patients before drug treatment and from ECs using Ingenuity Pathways Analysis. The top upstream regulator of genes in VL patient $CD4^+$ T cells, based on statistical significance, was IL-2 (Figure 4A). IL-2 was predicted to regulate genes with various immune functions located in the plasma membrane, cytoplasm and nucleus, as well as secreted $CD4^+$ T cell products (Figure 4B). Therefore, we next investigated the therapeutic potential of IL-2 for treating VL.

Targeting the high affinity IL-2R to enhance anti-parasitic immunity during established experimental VL

First, we used a pre-clinical model of VL caused by infection of C57BL/6 with *L. donovani*. Previous work identified combinations of recombinant IL-2 and anti-IL-2 mAbs that could selectively target high or low-affinity IL-2 receptors [15]. We chose to target the high-affinity IL-2R during established infection using recombinant IL-2 complexed with the JES6.1A12 anti-IL-2 mAb (IL-2Jc) because activated $CD4^+$ T cells express the high affinity IL-2R [7] and play a critical role in the control of parasite growth in this model [22, 24]. Infected mice were treated on days 14 and 21 post-infection (p.i.), and the impact on parasite burden was assessed at day 28 p.i.. Animals treated with the IL-2Jc had significantly lower liver and spleen parasite burdens, compared to control mice treated with vehicle (saline), JES6.1A12 anti-IL-2 mAb alone or recombinant IL-2 alone (Figure 5A). However, no changes in NK, $CD4^+$ T or $CD8^+$ T cell numbers, or their ability to produce IFNγ, in the liver and spleen at the time of parasite burden measurement (day 28 p.i.) was found (data not shown), suggesting a potent, but transient effect of IL-2Jc administration.

To establish whether $CD4^+$ T cells were needed for the therapeutic effects of IL-2Jc, we depleted this cell population during IL-2Jc treatment, and found the positive therapeutic response was lost (Figure 5B). Although this result doesn't show $CD4^+$ T cells were a direct target for IL-2Jc treatment, it indicates $CD4^+$ T cells were critical for the anti-parasitic effect

of IL-2Jc in this preclinical model of VL (Figure 5B). Treg cells also express high levels of the high affinity IL-2R and can be an important cell target for IL-2/antibody complexes [7, 15]. Therefore, we next treated *L. donovani*-infected B6.Foxp3-DTR mice with DT to deplete Treg cells during IL-2Jc treatment to test whether they influenced treatment outcome. However, we found no significant impact of Treg cell depletion on the anti-parasitic effects of IL-2Jc treatment, compared to controls (Figure 5C). Together, these data establish the therapeutic potential of targeting the high affinity IL-2R in experimental VL, and identify conventional $CD4^+$ T cells as critical for the anti-parasitic effects of this treatment.

IL-2 improves anti-parasitic pro-inflammatory, but not regulatory responses in VL patient blood cells.

We next tested used recombinant human IL-2 in an *ex vivo* antigen-stimulated whole blood assay [17], rationalising that the relatively short-term nature of these assays (<24 hours) would not require the extended half-life of cytokine activity provided by the cytokine complexed with mAb. When blood from VL patients was stimulated with parasite antigen (SLA), we found a significant increase in IFNγ and IL-10 production, compared to blood cultured without antigen (Figure 6A), as previously reported [18]. The addition of IL-2 to blood cultures resulted in a significant increase in antigen-specific IFNγ, but not IL-10 production (Figure 6A), indicating a selective effect on IFNγ production. In contrast, addition of IL-2 alone to EC blood cells increased IFNγ and IL-10 production, independent of antigen stimulation (Figure 6B), possibly reflecting the autocrine growth promoting properties of IL-2 and the effect of the cytokine on circulating effector $CD4^+$ T cells. Thus, these data show that IL-2 can be used to selectively promote antigen-specific IFNγ production by blood cells from VL patients, thereby providing support for targeting the IL-2 signalling pathway for therapeutic advantage in this disease.

Discussion

We identified a molecular signature associated with peripheral blood $CD4^+$ T cells from VL patients, including reduced transcription of *GATA3*, *RORC* and *FOXP3* genes, associated with reduced frequencies of blood Th2, Th17 and Treg cells, respectively. We also found blood $TBET^+$ $CD4^+$ (Th1) cell frequency was reduced in VL patients 30 days after they commenced anti-parasitic drug treatment, despite no differences in $CD4^+$ T cell *TBX21* gene expression. Elevated levels of cytokines associated with Th1 and Th2 cell responses have been reported in VL patients [25-28], as well as in splenic transcripts encoding these cytokines in a hamster model of VL [29]. However, the human studies measured either plasma cytokine levels or focused on IL-10 as the main Th2 cytokine, which we now know is produced by Th1 cells in both humans and mice [28, 30, 31]. Thus, Th2 cell frequency in VL patient blood may have been over-estimated, although we cannot exclude the possibility they accumulate in infected tissues. Consistent with our finding of reduced frequencies of Th17 cells in VL patients, a report from the Sudan found that Th17 cell induction was strongly associated with protection against VL [32]. Hence, reduced Th17 cell frequencies in Indian VL patients may contribute to disease development. Studies on Treg cells in VL patients have been inconsistent [33-35], and this is in part attributed to different markers used to define these cells. Here, we relied only on FOXP3 expression to identify Treg cells, and further studies using a more definitive range of human Treg cell markers are needed to establish their role in VL patients.

We also found increased expression of immune checkpoint molecule genes in VL patient $CD4^+$ T cells, including LAG3, CD38 and CTLA4, all of which can influence $CD4^+$ T cell behaviour [36]. In addition, we found decreased expression of CCR4 and CCR6 which promote migration of $CD4^+$ T cells to the skin and liver, respectively [37, 38]. Given parasites reside in both tissues, impaired $CD4^+$ T cell migration caused by their down-regulation may contribute to establishment and/or persistence of infection in these sites. Furthermore, decreased expression of CD40L by VL patient $CD4^+$ T cells is likely to impact their ability to help dendritic cells, B cells and other CD40-expressing immune cells perform their functions [39]. Similarly, decreased expression of IFNγR1 by VL patient $CD4^+$ T cells may impact the maintenance of Th1 cells during infection, and contribute to the altered composition of $CD4^+$ T cell subsets, as well as expression of interferon-regulated genes and associated

immune functions [40]. However, increased expression of pro-inflammatory molecules such as IFNγ was found, suggesting VL patient blood comprises a heterogeneous population of $CD4^+$ T cells with different functions, possibly reflecting genetic diversity amongst patients and ECs, and different histories of pathogen exposure.

IL-2 was identified as a major regulator of VL patient $CD4^+$ T cell genes, and given our desire to target $CD4^+$ T cells, we decided to target the trimeric, high affinity IL-2R expressed by activated $CD4^+$ T cells in mice. In C57BL/6 mice, the liver is a site of acute, resolving infection where highly effective anti-parasitic $CD4^+$ T cells develop, while the spleen is a site of chronic infection characterised by dysfunctional $CD4^+$ T cells responses [41]. Previous studies demonstrated the utility of IL-2Jc in treating inflammation and autoimmune disease in mice [42, 43]. Our results showed that IL-2Jc can also be used for therapeutic advantage in an experimental model of VL with anti-parasitic effects in both liver and spleen. As expected, the anti-parasitic effect of IL-2Jc was dependent on $CD4^+$ T cells. Despite the potential for IL-2Jc to expand Treg cells [15], we found no impact of this regulatory T cell subset in IL-2Jc-treated animals, suggesting conventional, activated $CD4^+$ T cells were the main target of this treatment. It will be useful in future to investigate the potential for adjunctive therapy with conventional anti-parasitic drugs, as this is the likely way such a treatment would be used for VL patients.

Previous studies using whole blood transcriptional profiling found IL-2 activation and signalling pathways were down-regulated in VL patients infected with *L. infantum* [44], suggesting these pathways may be differentially regulated in different lymphocyte subsets. An earlier study also showed the addition of recombinant human IL-2 to Indian VL patient PBMCs stimulated with parasite antigen didn't rescue proliferative responses [45]. Although cell proliferation wasn't measured in our studies, we showed exogenous IL-2 enhanced parasite-specific IFNγ production by VL patient blood cells. Remarkably, there was limited antigen-specific effect on IL-10 production, possibly reflecting differential expression of the trimeric IL-2R on IFNγ- and IL-10-producing $CD4^+$ T cell populations. The impact of IL-2 on the expression of immunoregulatory/exhaustion markers should be investigated in future studies. Nevertheless, these results indicate that selective improvement of IFNγ production by $CD4^+$ T cells can be achieved in VL patients without accompanying IL-10 production and associated suppressive effects on anti-parasitic immunity [18, 30]. There have been

significant advancements in engineering IL-2 for extended half-life and targeting IL-2R expressed by specific immune cell subsets [46]. Future studies should test the therapeutic potential of these next-generation IL-2 molecules in VL.

In summary, we identified a distinct pattern of gene expression in $CD4^+$ T cells from VL patients. No clear association with any specific $CD4^+$ T cell subset was recognized, although, we found a transcriptional signature consistent with our finding of reduced Th2, Th17 and Treg cell frequencies in the blood of VL patients after drug-treatment. IL-2 was identified as a major upstream regulator of $CD4^+$ T cells from VL patients and the therapeutic potential of this cytokine was shown. Future studies should test recently developed IL-2 molecules with favourable therapeutic properties that allow safe targeting of specific $CD4^+$ T cell subsets with anti-parasitic activity.

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Figure legends

Figure 1. Nanostring analysis of immune-related genes in CD4$^+$ T cells reveal more up- than down-regulated genes. The volcano plots show the distribution of immune-related genes in comparisons between visceral leishmaniasis (VL, n=27) patients during infection (D0) and VL patients after treatment (DIS) (A), D0 and endemic controls (EC, n=15) (B), and DIS and EC (C). Genes were determined to be up-regulated or down-regulated in the condition listed first in the title above each panel. Vertical lines in panels indicate absolute $\log_2$FC = 0.5 and the horizontal lines indicate -$\log_{10}$(p-value FDR) = 1.3 (i.e. FDR < 0.05). Genes of interest that were found to be differentially expressed (-$\log_{10}$(false discovery rate; FDR > 1.3) i.e. FDR < 0.05) are indicated.

Figure 2. Changes in peripheral blood CD4$^+$ T cell subsets. The FACS plots show the gating strategy used to identify TBET$^+$, GATA3$^+$, RORCγ$^+$ and FOXP3$^+$ CD4$^+$ T cells (A), and the frequency of these subsets amongst CD4$^+$ T cells between visceral leishmaniasis (VL) patients during infection (D0, n = 7) VL patients after treatment (DIS, n = 7) and endemic controls (EC, n = 6), Median + Min and Max (B), **=p<0.01, *=p<0.05, Wilcoxon matched-pairs signed rank test.

Figure 3. Changes in immune molecule expression by CD4$^+$ T cells. The FACS plots show the gating strategy used to identify CD38$^+$, CTLA4$^+$, CCR6$^+$, CCR4$^+$, CD40L$^+$, IFNγR1$^+$ and CD96$^+$ CD4$^+$ T cells (A), and the frequency of these subsets amongst CD4$^+$ T cells between visceral leishmaniasis (VL) patients during infection (D0, n = 6) VL patients after treatment (DIS, n = 6) and endemic controls (EC, n = 6), Median + Min and Max (B), **=p<0.01, *=p<0.05, Wilcoxon matched-pairs signed rank test.

Figure 4. IL-2 is the top upstream regulator of immune-related genes in visceral leishmaniasis patients. The table lists the top 12 upstream regulators (sorted by *p*-value of overlap) that were identified by Ingenuity Pathway Analysis (A). The network shows all up-regulated (red) and down-regulated (green) genes within the dataset which were predicted to be regulated by IL-2, and illustrates the predicted relationship between IL-2 and these genes (B).

Figure 5. Mouse IL-2/antibody complexes stimulate CD4$^+$ T cell-dependent anti-parasitic immunity in mice infected with *Leishmania donovani*. C57BL/6 mice were infected with

parasites for 14 days and then administered saline, anti-IL-2 mAb (JES6.1A12), recombinant mouse IL-2 (rmIL-2) or anti-IL-2 mAb complexed with rmIL-2 (IL-2Jc) on days 14 and 21 p.i., as indicated, and parasite burdens in the liver and spleen were measured 14 days later (day 28 p.i.) (A). *L. donovani*-infected mice receiving saline or IL-2Jc were treated with control mAb (MAC5) or anti-CD4 mAb every 3 days, as indicated, over the same time period and parasite burdens in the liver and spleen were measured at day 28 p.i. (B). *L. donovani*-infected B6.Foxp3.DTR mice receiving saline or IL-2Jc were treated with saline or diphtheria toxin (DT) every 3 days, as indicated, over the same time period and parasite burdens in the liver and spleen were measured at day 28 p.i. (C). All values are mean ± SEM of at least 2 independent experiments, n=5 mice per group. Mean ± SEM, *p<0.05, **p<0.01, and ***p<0.001; significance assessed by one-way ANOVA.

Figure 6. Human IL-2 stimulate antigen-specific IFNγ, but not IL-10 production by whole blood cells from visceral leishmaniasis (VL) patients. Antigen-specific IFN-γ and IL-10 production was measured in whole blood cells from VL patients on admission to clinic (n=8-26) (**A**) and from endemic controls (n=10-24) (B) cultured for 24 hours with media alone (NIL), soluble leishmania antigen (SLA), recombinant human IL-2 (IL-2) or SLA + IL-2, as indicated. Median + Min and Max, *p<0.05, **p<0.01, and ***p<0.001; significance assessed by Wilcoxon matched-pairs signed rank test.

Figure 1

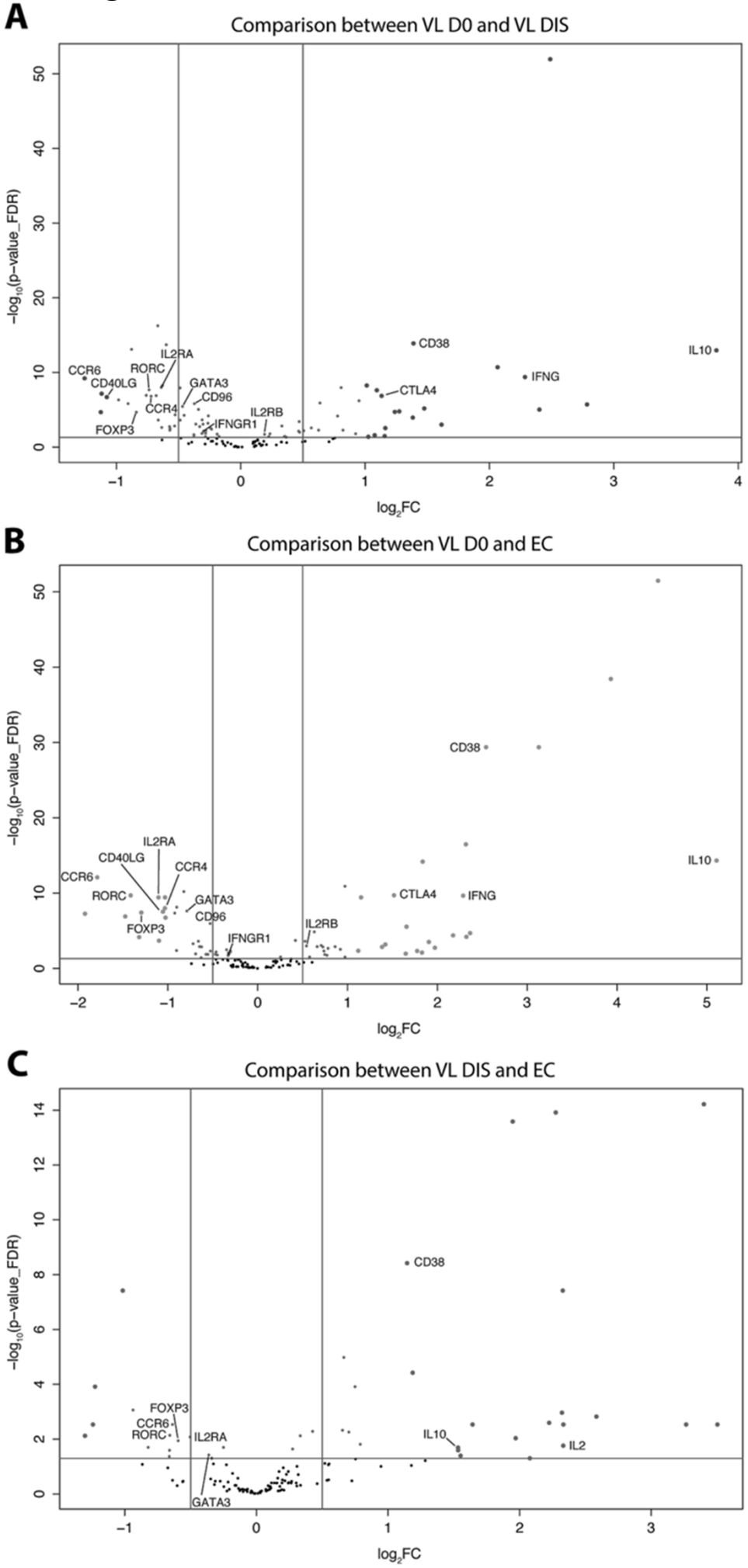
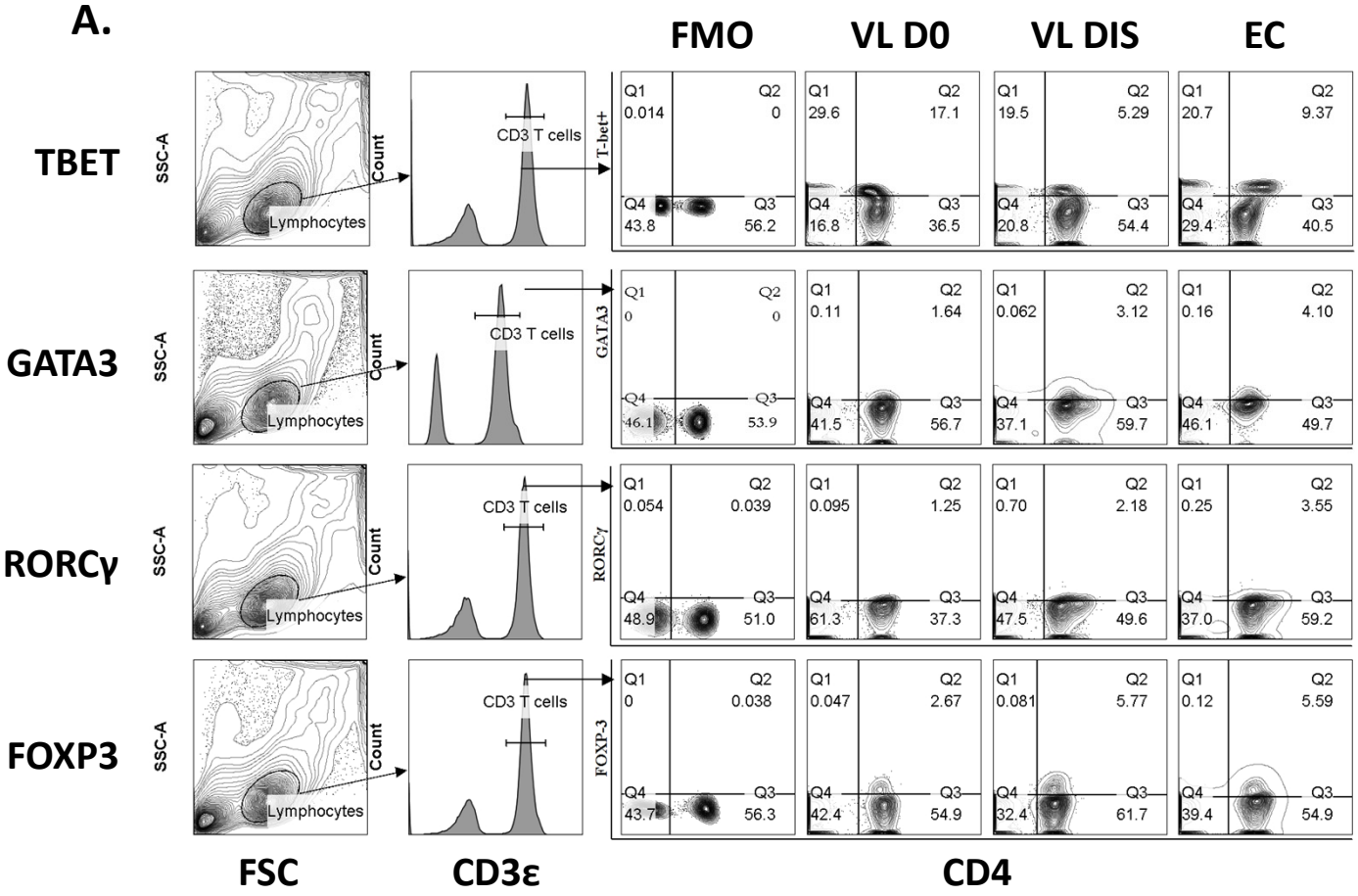


Figure 2



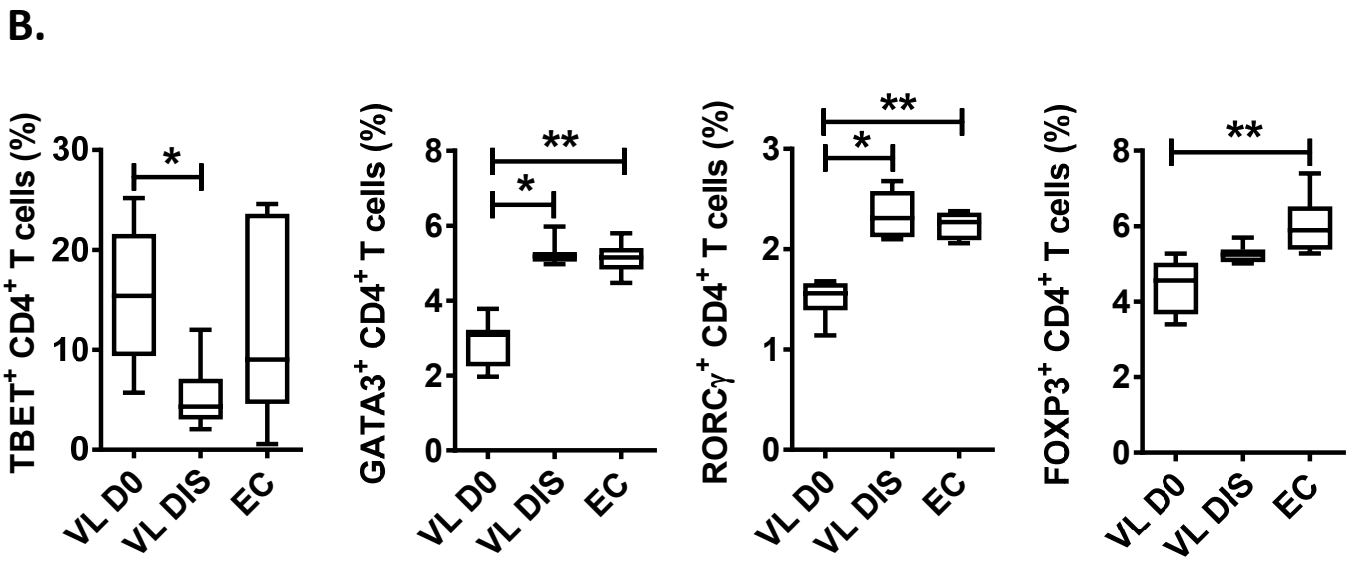
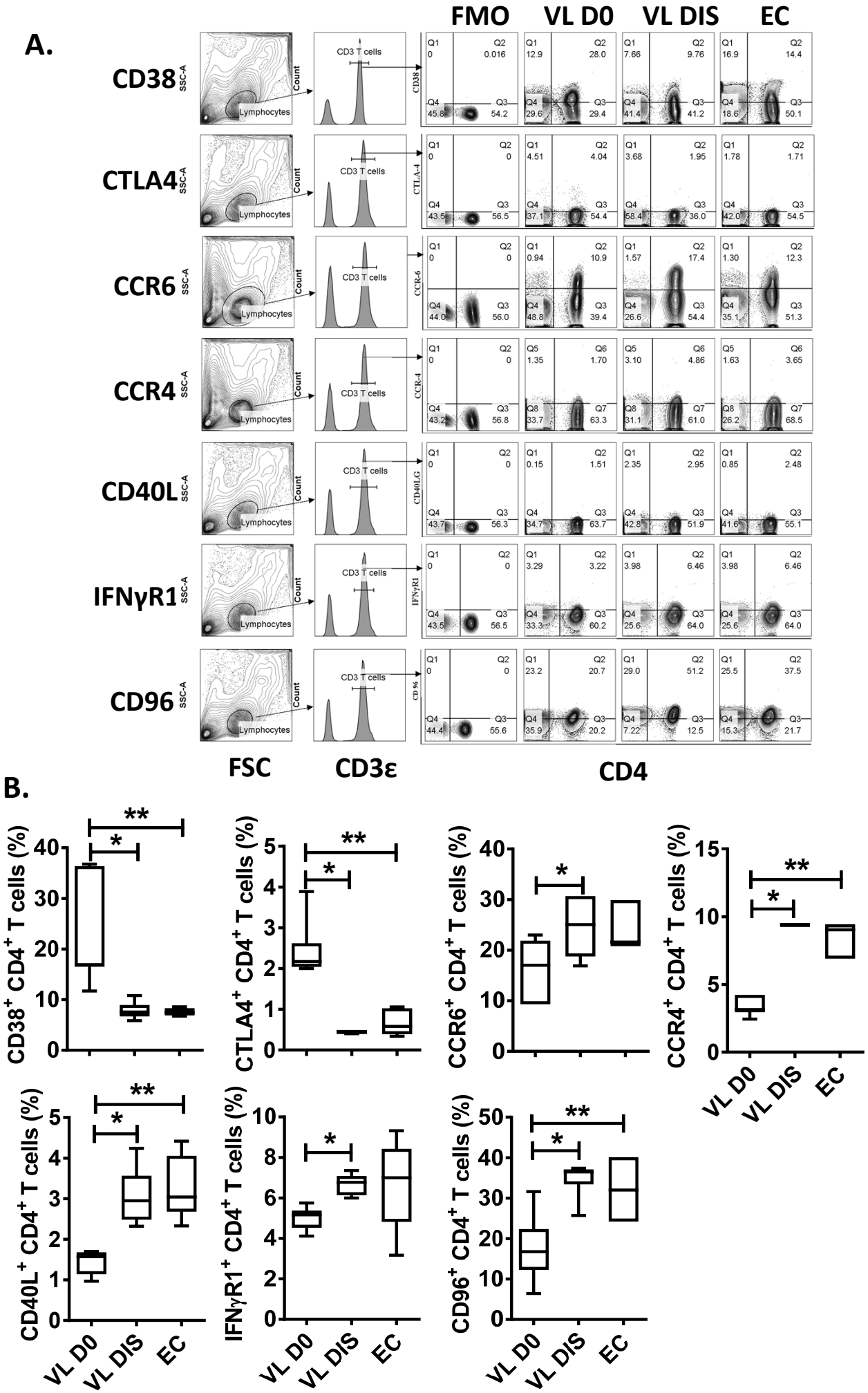


Figure 3



A

Figure 4

Top 12 upstream regulators

Upstream regulator	Activation *z*-score	*p*-value of overlap
IL2	2.337	1.05e-65
TCR	1.365	5.07e-61
IL21	3.307	9.19e-61
CD28	2.154	6.20e-58
IL12 (complex)	2.015	1.02e-55
IL27	2.142	1.17e-53
CD3	1.464	1.30e-53
IL10	0.518	6.53e-48
IL4	1.026	4.77e-47
IL15	2.241	8.22e-47
TBX21	2.537	5.48e-46
IL12 (family)	0.739	4.72e-45

B

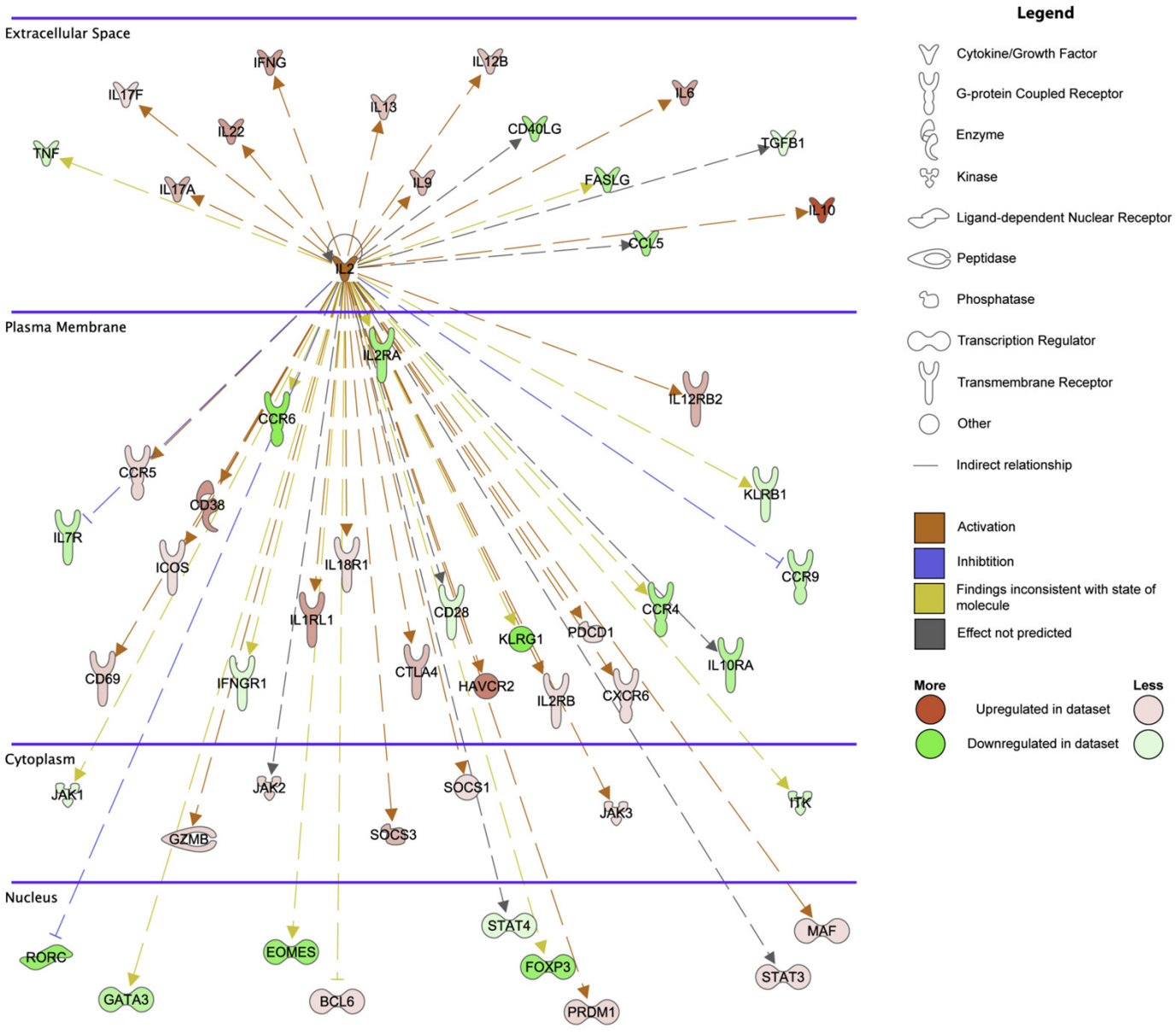


Figure 5

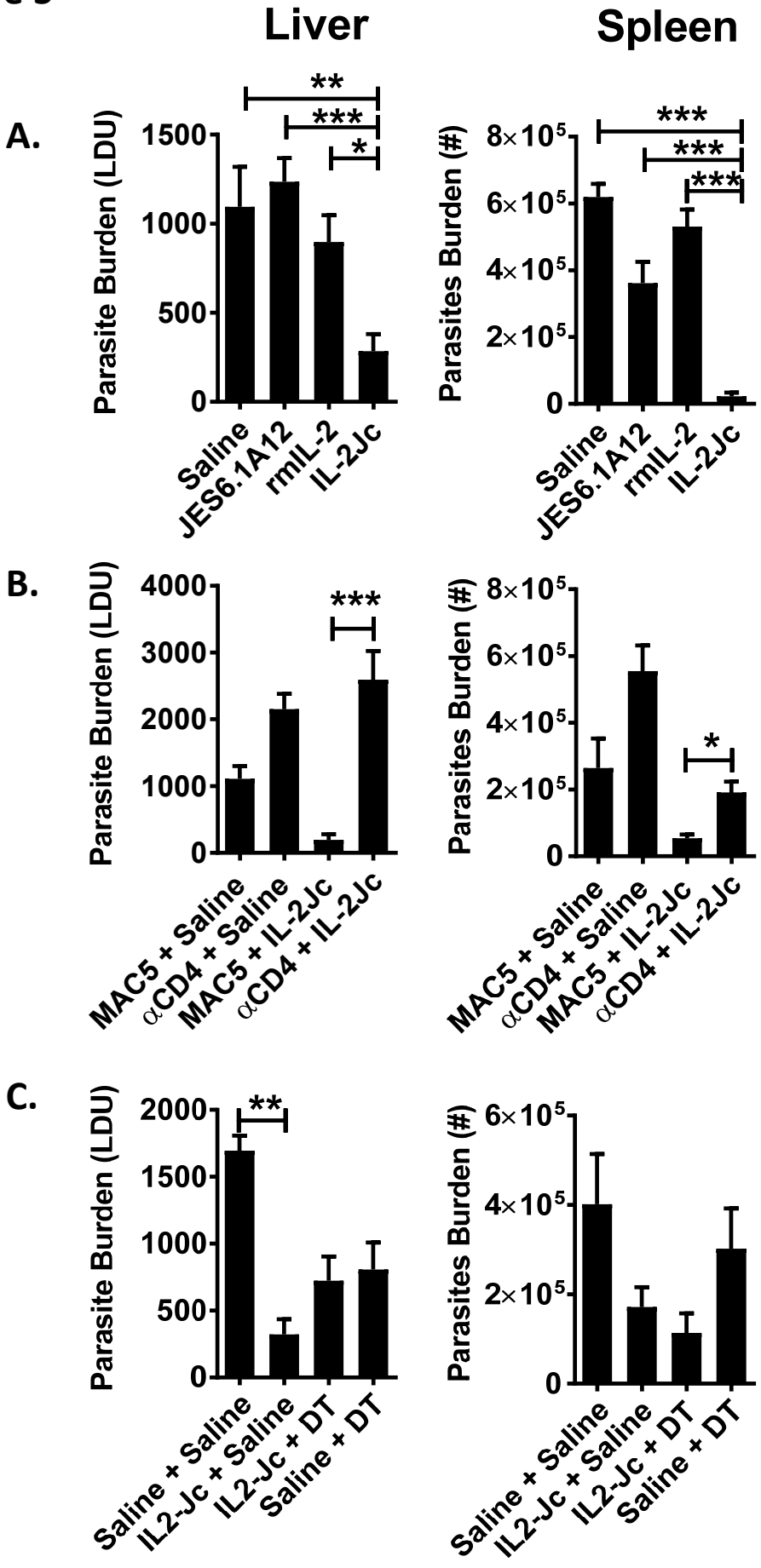


Figure 6