


Hepatocyte CD1d protects against liver immunopathology in mice with schistosomiasis japonica

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INTRODUCTION

Schistosomiasis is a neglected tropical disease and affects over 250 million people worldwide. The schistosome species considered to be of major medical importance are *Schistosoma mansoni* (*S. mansoni*), *Schistosoma japonicum* (*S. japonicum*) and *Schistosoma haematobium*. During *S. mansoni* and *S. japonicum* infections, the host liver is the

Summary

Schistosomiasis is a neglected tropical disease with over 250 million people infected worldwide. The main clinically important species *Schistosoma mansoni* (*S. mansoni*) and *Schistosoma japonicum* (*S. japonicum*) cause inflammatory responses against tissue-trapped eggs, resulting in formation of granulomas mainly in host liver. Persistent granulomatous response results in severe fibrosis in the liver, leading to irreversible impairment of the liver and even death of the host. CD1d, a highly conserved MHC class I-like molecule, is expressed by both haematopoietic and non-haematopoietic cells. CD1d on antigen-presenting cells (APCs) of haematopoietic origin presents pathogen-derived lipid antigens to natural killer T (NKT) cells, which enables them to rapidly produce large amounts of various cytokines and facilitate CD4⁺ T helper (Th) cell differentiation upon invading pathogens. Noteworthy, hepatocytes of non-haematopoietic origin have recently been shown to be involved in maintaining liver NKT cell homeostasis through a CD1d-dependent manner. However, whether hepatocyte CD1d-dependent regulation of NKT cell homeostasis also modulates CD4⁺ Th cell responses and liver immunopathology in murine schistosomiasis remains to be addressed. Here, we show in mice that CD1d expression on hepatocytes was decreased dramatically upon *S. japonicum* infection, accompanied by increased NKT cells, as well as upregulated Th1 and Th2 responses. Overexpression of CD1d in hepatocytes significantly decreased local NKT numbers and cytokines (IFN- γ , IL-4, IL-13), concomitantly with downregulation of both Th1 and Th2 responses and alleviation in pathological damage in livers of *S. japonicum*-infected mice. These findings highlight the potential of hepatocyte CD1d-targeted therapies for liver immunopathology control in schistosomiasis.

Keywords: CD1d; hepatocyte; schistosomiasis; immunopathology.

principal site that is affected. The adult parasites reside in mesenteric veins and lay eggs. Many of the eggs are carried by blood flow into liver and trapped in the microvasculature of the liver. Consequently, immunopathological responses against trapped eggs in liver lead to formation of granulomas, which are progressively replaced by fibrotic deposits eventually resulting in cirrhosis, portal hypertension and even death of the host.^{1–4}

Abbreviations: *S. mansoni*, *Schistosoma mansoni*; *S. japonicum*, *Schistosoma japonicum*; APCs, antigen-presenting cells; NKT, natural killer T cells; Th, CD4⁺ T helper cells; IFN- γ , interferon gamma; IL-4, interleukin-4; IL-13, interleukin-13; AAV8, adeno-associated virus serotype 8; FCM, flow cytometry; MNCs, mononuclear cells

CD1d is a highly conserved MHC class I-like molecule and expressed by both professional antigen-presenting cells (APCs) of haematopoietic origin (dendritic cells, macrophages and B cells) and non-haematopoietic cells (hepatocytes, epithelial cells, adipocytes, etc.).^{5–7} In particular, CD1d mostly presents lipid antigens to natural killer T (NKT) cells.⁸ Studies show that CD1d on APCs presents pathogen-derived lipid antigens to NKT cells, which enables them to rapidly produce large amounts of various cytokines and facilitate CD4⁺ T helper (Th) cell differentiation upon invading pathogens.^{9,10} During murine schistosomiasis, NKT cell subset is one of the important mediators of hepatic inflammation during schistosome infection. Schistosome-derived lipid antigens are presented to NKT cells by CD1d on APCs, leading to amplification of Th2 responses and egg-induced pathology in liver.^{11,12}

Noteworthy, CD1d is highly expressed on hepatocytes and a recent study has revealed an unanticipated role of parenchymal cells that hepatocyte CD1d can control local NKT cell numbers and maintain immunological homeostasis in the liver through directly inducing NKT cell apoptosis.^{13,14} However, whether hepatocyte CD1d-dependent regulation of NKT cell homeostasis has modulating effects on CD4⁺ Th cell responses and immunopathology during murine schistosomiasis remains to be addressed.

Therefore, we aimed to determine the function and mechanism of hepatocyte CD1d in hepatic schistosomiasis. Here, we found that *S. japonicum* infection leads to a dramatic decrease in hepatocyte CD1d expression. Overexpression CD1d in hepatocytes significantly decreased local NKT numbers and Th1/Th2 cytokines (IFN- γ , IL-4, IL-13) in liver, downregulated both Th1 and Th2 responses and attenuated liver immunopathological damage in *S. japonicum*-infected mice. Thus, hepatocyte CD1d is suggested as a promising regulator of liver immunopathology in hepatic schistosomiasis.

MATERIALS AND METHODS

Ethics statement and mice

All the animals were handled in strict accordance with Regulations for the Administration of Affairs Concerning Experimental Animals. All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of Nanjing Medical University (Approval Number: IACUC-1905064).

Specific pathogen-free (SPF) 6- to 8-week-old male mice were purchased from the Animal Center of Nanjing Medical University. All mice were maintained in an SPF breeding facility with standard environment conditions (ambient temperature, 22°C; humidity, 40%; 12-h light–dark cycle). All efforts were made to minimize suffering.

Infection of mice with *S. japonicum*

Mice were infected percutaneously with 12 cercariae of the Chinese mainland strain of *S. japonicum* from infected snails (*Oncomelania hupensis*), which were purchased from the Jiangsu Institute of Parasitic Diseases.

Production of CD1d-expressing AAV8 vector and in vivo delivery methods

A recombinant adeno-associated viral serotype 8 vector (AAV8) containing mouse *CD1d1* gene sequence and an empty AAV8 vector control were purchased from Hanbio Biotechnology. The recombinant vector was named as AAV8-CMV-m-CD1d1-3xflag-ZsGreen (AAV-CD1d) or AAV8-ZsGreen (AAV-Ctrl).

A week before *S. japonicum* cercariae infection, each mouse was injected via tail vein containing 1×10^{11} particles of either AAV-CD1d or AAV-Ctrl vectors. At 8 weeks post-infection, mice were killed, and spleens and liver samples were collected for analysis.

Western blot

Western blot analysis was performed according to previous standard procedures. Liver tissues or isolated primary hepatocytes were lysed with RIPA buffer (Invitrogen). Total protein lysates were heat-denatured, separated by 12% SDS-PAGE, transferred onto nitrocellulose membranes (Whatman, Maidstone) and then detected with the following primary antibodies: anti-CD1d (Cat. No. AF4884, R&D Systems), anti-IL-33 (clone Nussy-1, Abcam), anti- β -actin (clone D6A8, CST) and anti-GAPDH (clone D16H11, CST). Secondary antibodies used in this study are as follows: HRP-linked goat anti-rabbit IgG (CST) and HRP-linked rabbit anti-sheep IgG (BioVision). Protein bands were detected using ImmunoStar HRP Substrate (Bio-Rad), digitally captured with the Bio-Rad Gel Doc XR System (Bio-Rad), and quantified with the ImageJ software (NIH; <http://imagej.nih.gov/ij>).

Quantitative real-time PCR (qPCR)

Tissue or cell samples were homogenized or lysed in TRIzol™ Reagent (Invitrogen), and total RNA was isolated with a phenol–chloroform extraction. Isolated RNA (1 μ g) from each sample was reverse-transcribed using the SuperScript III First-Strand cDNA Synthesis System (Invitrogen). RT-PCRs were performed on the ABI PRISM 7300 Real-Time PCR System (Applied Biosystem) with FastStart SYBR Green Master Mix (Roche Applied Science). Gene expression levels were calculated using the following formula: Fold change = $2^{-\Delta\Delta Ct}$, $\Delta\Delta Ct = \Delta Ct_{\text{sample}} - \Delta Ct_{\text{control}}$, and $\Delta Ct = Ct_{\text{target}} - Ct_{\text{gapdh}}$. Primers used were as follows:

- Cd1d1, forward, 5'-GCAGCCAGTACGCTCTTTTC-3', and reverse, 5'-ACAGCTTGTTTCTGGCAGGT-3';
- PU.1, forward, 5'-AGAAGCTGATGGCTTGGAGC-3', and reverse, 5'-GCGAATCTTTTCTTGCTGCC-3';
- Lef-1, forward, 5'-TCCTGAAATCCCCACCTTCT-3', and reverse, 5'-TGGGATAAACAGGCTGACCT-3';
- Sp1, forward, 5'-CTCCAGACCATTAACCTCAGTGC-3', and reverse, 5'-CAAGTAACGGCATGGAGGAC-3';
- GAPDH, forward, 5'-TGGTGAAGGTCGGTGTGAAC-3', and reverse, 5'-CCATGTAGTTGAGGTCAATGAAGG-3';
- IFN- γ , forward, 5'-TGCTGATGGGAGGAGATGTCT-3', and reverse, 5'-TGCTGTCTGGCCTGCTGTTA-3';
- IL-13, forward, 5'-CCTGGCTCTTGCTTGCCTT-3', and reverse, 5'-GGTCTTGTGTGATGTTGCTCA-3';
- IL-4, forward, 5'-ACAGGAGAAGGGACGCCAT-3', and reverse, 5'-GAAGCCCTACAGACGAGCTCA-3';
- IL-10, forward, 5'-ACTTTAAGGGTTACTTGGGTTGC-3', and reverse, 5'-ATTTTCACAGGGGAGAAATCG-3';
- TNF- α , forward, 5'-AACCACCAAGTGGAGGAGCAGCT-3', and reverse, 5'-GGAAGACTCCTCCCAGGTATATGG G-3'; and
- TGF- β 1, forward, 5'-ATGCTAAAGAGGTCACCCGC-3', and reverse, 5'-CCAAGTAACGCCAGGAATT-3'.

Serum ALT/AST analysis

The levels of serum ALT and AST were tested by Olympus AU2700 Chemical Analyzer (Olympus) according to the manufacturer's guide.

Flow cytometry (FCM) analysis

Mouse hepatic mononuclear cells (MNCs) were isolated using Percoll density gradient centrifugation as described previously.¹⁵ For NKT cell analysis, MNCs were surface-stained with CD3e-Percp-Cy5.5 (clone 145-2C11, BD Bioscience, San Diego, CA) and NK1.1-FITC (clone PK136, eBioscience). To analyse CD1d expression on macrophages, MNCs were surface-stained with CD11b-FITC (clone M1/70, BD Bioscience), F4/80-APC (clone BM8, eBioscience) and CD1d-PE (clone 1B1, Invitrogen). For CD1d analysis on dendritic cells (DCs), MNCs were surface-stained with F4/80-Percp-Cy5.5 (clone BM8, Invitrogen), MHCII-PE-Cy7 (clone M5/114.15.2, eBioscience), CD11c-APC (clone N418, eBioscience) and CD1d-PE (clone 1B1, Invitrogen).

Mouse hepatic lymphocytes were purified using lympholyte M (Cedarlane) according to the manufacturer's instructions. To analyse CD1d expression on T cells, cells were surface-stained with CD3e-Percp-Cy5.5 (clone 145-2C11, BD Bioscience) and CD1d-PE (Invitrogen).

To analyse CD1d expression on B cells, cells were surface-stained with CD19-FITC (clone 6D5, Invitrogen) and CD1d-PE (Invitrogen). To determine intracellular cytokine expression, cells were stimulated with 25 ng/ml of phorbol myristate acetate (PMA, Sigma-Aldrich) and 1 μ g/ml of ionomycin (Sigma-Aldrich) in the presence of 1 μ l/ml of GolgiStop (BD Bioscience). After 6 h, the cells were collected and surface-stained with CD3e-APC (clone 145-2C11, eBioscience) and CD4-FITC (clone GK1.5, BioLegend), and washed, fixed and permeabilized with Cytotfix/Cytoperm buffer (BD Bioscience). Next, the cells were intracellularly stained with PE-conjugated antibodies against IFN- γ -PE (clone XMGI.2, BioLegend) and IL-4-PE (clone 11B11, eBioscience). To analyse regulatory T cells, hepatic lymphocytes were surface-stained with CD3e-Percp-Cy5.5 (clone 145-2C11, BD Bioscience), CD4-FITC (clone GK1.5, BioLegend) and CD25-APC (clone PC 61.5, eBioscience). The cells were then permeabilized with cold Fix/Perm Buffer (eBioscience™ Foxp3/Transcription Factor Staining Buffer Set, Cat. No. 00-5523-00), and the Fc receptors were blocked with anti-mouse CD16/32 (Fc Block, Invitrogen). Finally, cells were stained with Foxp3-PE (clone FJK-16 s, eBioscience).

To analyse CD1d expression on primary hepatocytes from normal and infection mice, hepatocytes were prepared as below and cells were washed and then stained with PE-conjugated antibodies against CD1d (clone 1B1, Invitrogen) or the isotype control antibody (clone eB149/10H5, eBioscience).

Following staining, cells were examined using a FACSVerse instrument (BD Bioscience) and analysed using the FlowJo software (version 10.0.7; Tree Star).

Extraction of primary hepatocytes

Primary hepatocytes were separated as previously described.¹⁶ Briefly, mice were anaesthetized and their livers were perfused with HBSS containing 0.5 m EGTA via portal vein, followed by 0.05% collagenase IV digestive fluid. The digested liver suspension was filtered through a 200-gauge steel mesh, and the hepatocytes in the flow through were isolated by 40% Percoll.

Statistical analysis

Statistical analysis was performed using the SPSS program (version 26 for Windows, IBM Corp.). The comparisons of two group were analysed by Student's *t*-test, while multiple group analysis was carried out by one-way analysis of variance (ANOVA) followed by an LSD post hoc test. For all statistical analyses, *P* < 0.05 was considered significant.

RESULTS

CD1d expression dramatically decreases in liver of *S. japonicum*-infected mice

To examine CD1d expression in livers of *S. japonicum*-infected mice, RT-PCR and Western blot were performed, respectively. We found that infected mice livers showed a significant decline in CD1d mRNA levels as early as 1 week after *S. japonicum* infection and a more than 15-fold decline at 3 weeks post-infection, and thereafter remained at a very low level (Figure 1A). A corresponding significant, progressive decrease in CD1d expression was also shown by Western blotting starting from 1 week after infection (Figure 1B). These data demonstrated a dramatic decrease in hepatic CD1d expression in mice with *S. japonicum* infection.

Hepatocytes are the major contributor to the dramatic reduction in hepatic CD1d expression in *S. japonicum*-infected mice

Hepatocytes account for the vast majority of cells in liver and express a high level of CD1d among liver parenchymal cells.^{13,17} We investigated the expression of CD1d on hepatocytes from *S. japonicum*-infected mice. Results showed a sharp decline in CD1d expression on hepatocytes after *S. japonicum* infection (Figure 2A,B). To rule out non-specific experimental factors that may repress gene expression in hepatocytes, IL-33, which is an alarmin and increases after *S. japonicum* infection,¹⁸ was used as a control. Result showed that IL-33 expression increased (Figure S1). In addition, FCM analysis showed that surface expression of CD1d on hepatocytes

dramatically decreased in infected mice (Figure 2C). We also detected CD1d expression by FCM on some other cells of haematopoietic origin in the liver of *S. japonicum*-infected mice. Results showed no significant changes in CD1d expression on F4/80⁻CD11c⁺MHCII⁺ DCs, CD11b⁺F480⁺ macrophages and CD3⁺ T cells after *S. japonicum* infection (Figure 2D–F). However, CD19⁺ B cells presented a mild decline in CD1d expression after infection (Figure 2G). Collectively, these results suggest that hepatocytes are the major contributor to the dramatic reduction in hepatic CD1d expression in *S. japonicum*-infected mice.

Furthermore, hepatocytes from *S. japonicum*-infected mice showed a dramatic decrease in SP1 mRNA level and significant increase in both PU.1 and LEF-1 mRNA levels (Figure S2A–C), which are transcriptional activator (SP1) or repressors (PU.1 and LEF-1) of CD1d gene.^{19–21}

AAV8-mediated overexpression of CD1d leads to protection against pathological damage in *S. japonicum*-infected mouse livers

Given the dramatic decrease in CD1d expression observed in hepatocytes from *S. japonicum*-infected mice, we used an AAV8 vector, which is proven to have high affinity for hepatocytes and achieves $\approx 95\%$ hepatocyte transduction rate,^{22–24} to overexpress CD1d in hepatocytes to investigate its role in pathological damage during schistosomiasis. The detailed experimental design is shown in Figure 3A. Nine weeks after tail vein injection of AAV8 vector encoding CD1d gene, efficient CD1d overexpression was mainly observed in the liver but not detected in the spleen (Figure 3B,C). *S. japonicum*-infected mice with CD1d overexpression showed decreased size of isolated

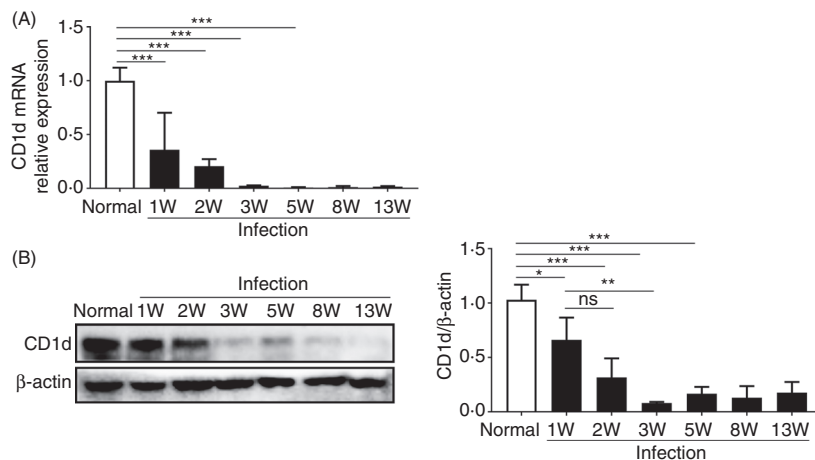


Figure 1. CD1d expression dramatically decreases in liver of *S. japonicum*-infected mice. (A and B) CD1d mRNA and protein expression levels in liver samples of normal or *S. japonicum*-infected mice (1, 2, 3, 5, 8 and 13 weeks post-infection) were analysed by RT-PCR (A) and Western blot (B), respectively. The graphs (B) show the quantification of the Western blot. Data are means \pm SD of 4 mice and representative of three independent experiments. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$; ns, not significant.

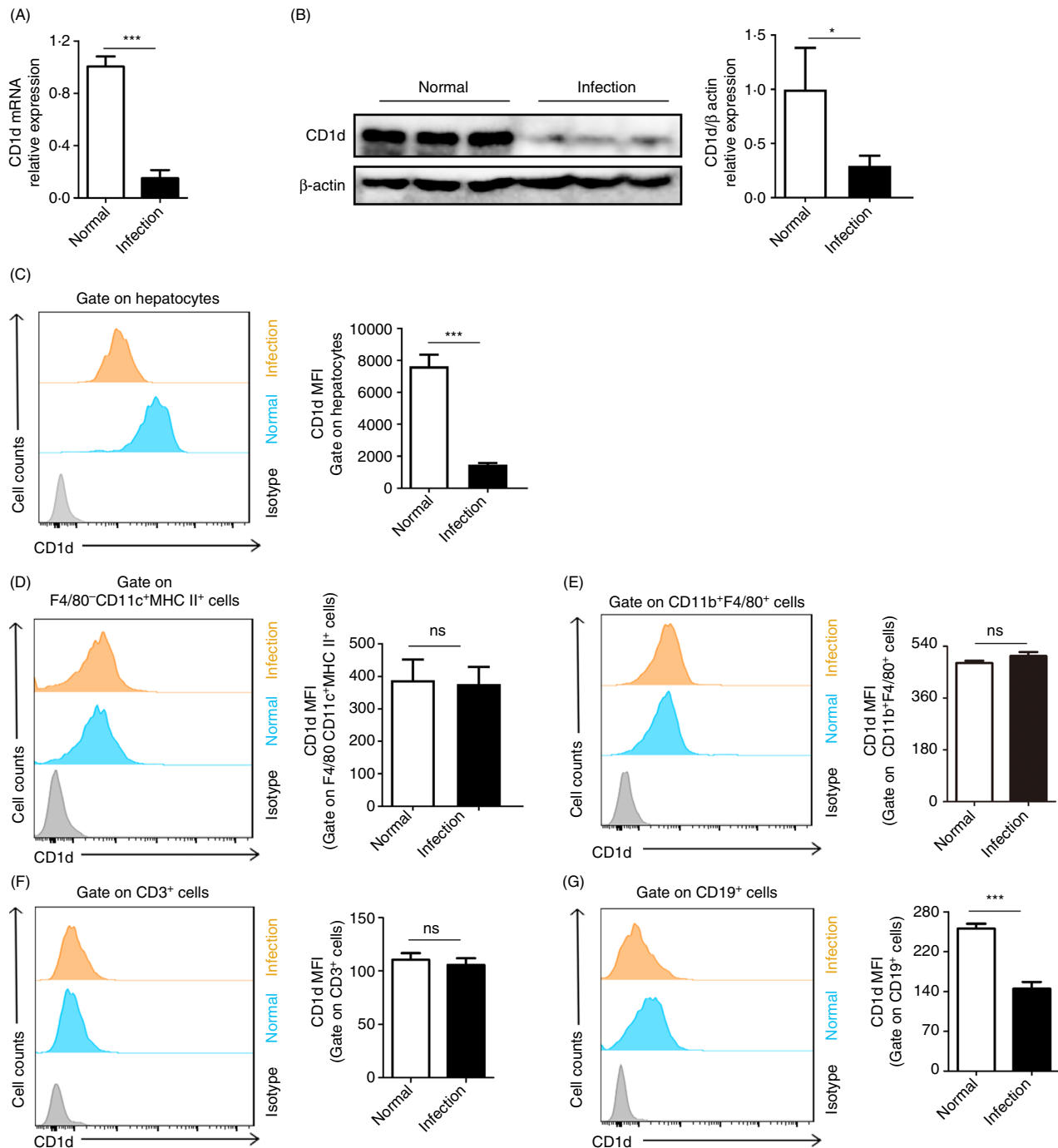


Figure 2. Hepatocytes are the major contributor to the dramatic reduction in hepatic CD1d expression in *S. japonicum*-infected mice. (A and B) CD1d mRNA and protein expression levels in hepatocytes from livers of normal or *S. japonicum*-infected (8 weeks post-infection) mice were determined by RT-PCR (A) and Western blot (B), respectively. Representative images of Western blots are shown, and protein level of CD1d normalized against β -actin was quantified by ImageJ software. (C–G) CD1d expression on hepatocytes (C), F4/80⁻CD11c⁺MHCII⁺ DCs (D), CD11b⁺F480⁺ macrophages (E), CD3⁺ T cells (F) and CD19⁺ B cells (G) from livers of normal or *S. japonicum*-infected (8 weeks post-infection) mice were analysed by FCM, and representative histograms are shown. The bar graphs show the average MFI of CD1d expression in each cell population. Data are means \pm SD from 4 mice per group, and similar results were obtained in two independent experiments. * $P < 0.05$ and *** $P < 0.001$; ns, not significant.

hepatic granulomas (Figure 3D,E). In addition, both ALT and AST levels were significantly reduced in infected mice with AAV8-CD1d injection (Figure 3F). These results

suggest that hepatocyte CD1d may be involved in protection against pathological damage in *S. japonicum*-infected mice.

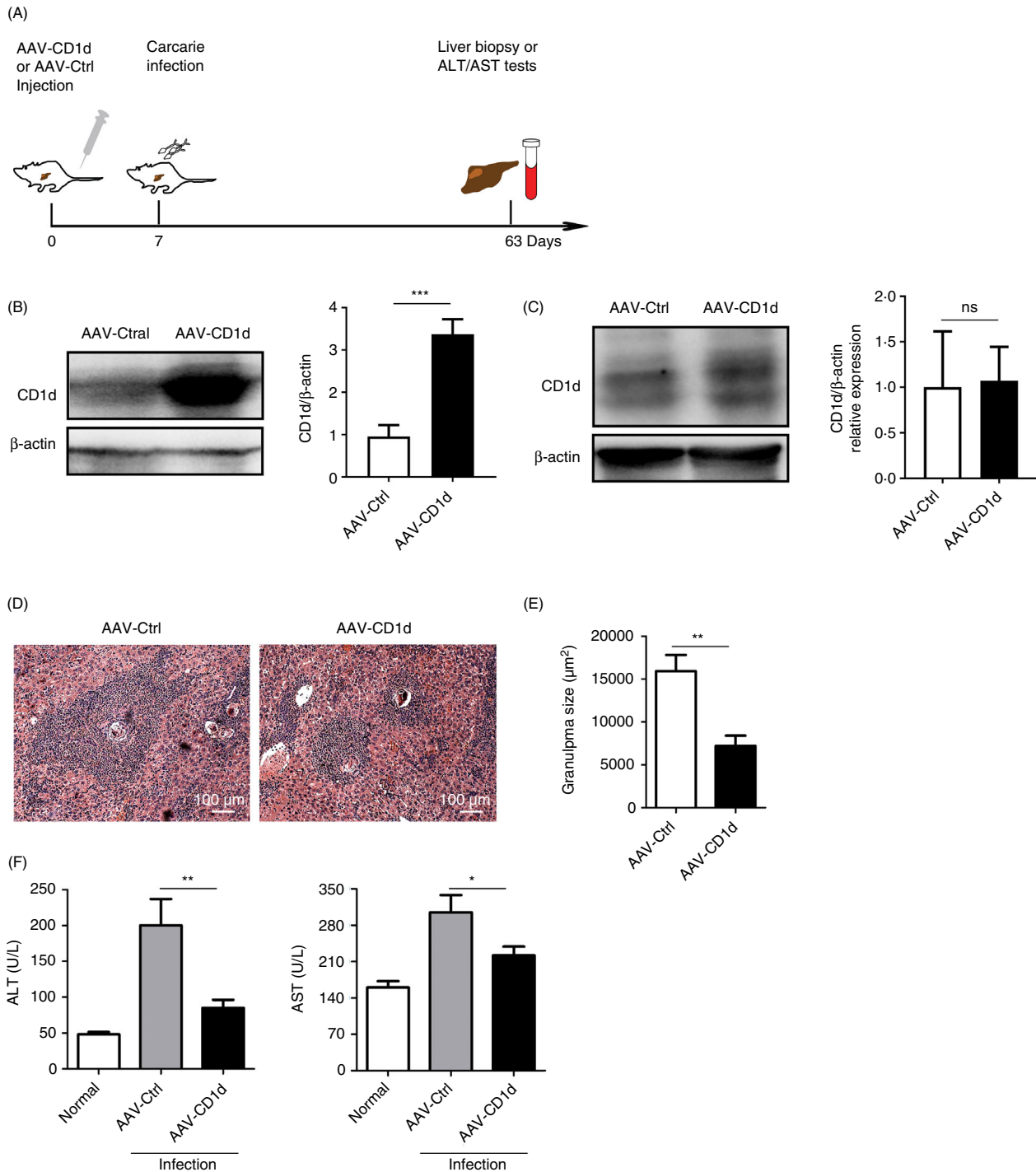


Figure 3. AAV8-mediated overexpression of CD1d leads to protection against pathological damage in *S. japonicum*-infected mice. (A) Experimental design for AAV8 vector (AAV-CD1d or AAV-Ctrl) injection, cercariae infection and further analysis. (B–C) Effect of AAV vectors on CD1d protein expression in livers (B) and spleens (C) of mice injected with AAV-CD1d or AAV-Ctrl was analysed by Western blot. The graphs show the quantification of the Western blot. (D) Representative liver sections of *S. japonicum*-infected mice injected with AAV-CD1d or AAV-Ctrl were H&E-stained to show granulomas (original magnifications, ×100). (E) Granuloma areas with a single egg were measured. (F) Levels of alanine transaminase (ALT) and aspartate transaminase (AST) in the serum of mice with injection of AAV-CD1d or AAV-Ctrl were detected by an automatic biochemical analyser. Data are means ±SD from 5 mice per group and representative of two independent experiments. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$; ns, not significant.

Decreased NKT cells accompanying downregulation of both Th1 and Th2 cytokines in CD1d-overexpressing liver of *S. japonicum*-infected mice

Following *S. japonicum* infection, despite decreased frequency of CD3⁺NK1.1⁺ NKT cells (Figure 4A), the absolute numbers of mononuclear cells (MNCs) and NKT cells were increased in the livers of infected mice (Figure 4B). Hepatocyte CD1d can maintain immunological homeostasis in the liver by controlling local NKT cell numbers.¹⁴ We next investigated whether overexpression of CD1d affects the homeostasis of hepatic NKT cells in *S. japonicum*-infected mice. FCM analysis revealed significantly decreased frequencies and absolute numbers of hepatic NKT cells in *S. japonicum*-infected mice with AAV-CD1d injection compared with those in *S. japonicum*-infected mice with AAV vector injection, but without changes in the total numbers of liver MNCs between groups (Figure 4C,D). As expected, the decrease in NKT cells was mainly in the liver but not in the spleen of AAV-CD1d-injected mice (Figure S3).

Following activation, NKT cells can facilitate CD4⁺ T helper (Th) cell differentiation by rapidly releasing large amounts of various cytokines. The type and quantity of cytokine release differs depending on disease or tissue context.^{7,25} We further explored the changes in cytokine profile in the liver by RT-PCR. Interestingly, except for TNF- α , both Th1 (IFN- γ) and Th2 (IL-4 and IL-13) cytokines were found to be significantly decreased in the liver of *S. japonicum*-infected mice with AAV-CD1d injection (Figure 4E). Additionally, immunoregulatory cytokine IL-10 showed a trend towards increase that was not statistically significant, whereas TGF- β level did not show any change (Figure 4E). Together, these results suggest that hepatic CD1d regulates liver NKT cell homeostasis, which may lead to impaired inflammatory responses to schistosome eggs in liver of *S. japonicum*-infected mice.

Both Th1 and Th2 cell responses in livers are impaired in *S. japonicum*-infected mice with CD1d overexpression

The CD4⁺ T cell subsets are involved in the regulation of schistosomiasis progression, and their differentiation is tightly regulated by the local cytokine profile.²⁶ Following *S. japonicum* infection, the frequencies and absolute numbers of Th1 (IFN- γ ⁺), Th2 (IL-4⁺) and Treg (CD25⁺Foxp3⁺) cells in hepatic CD3⁺CD4⁺ T cells apparently increased (Figure 5A). In accordance with cytokine profile changes shown in Figure 4E, results further showed decreased frequencies and absolute numbers of Th1 (IFN- γ ⁺) and Th2 (IL-4⁺) cells in hepatic CD4⁺ T cells of *S. japonicum*-infected mice with CD1d overexpression (Figure 5B,C), whereas the frequencies and absolute

numbers of Treg (CD25⁺Foxp3⁺) cells in hepatic CD4⁺ T cells did not show any significant change (Figure 5D). These results indicate that hepatic CD1d is important in regulating both Th1 and Th2 cell responses during *S. japonicum* infection.

DISCUSSION

After *S. mansoni* or *S. japonicum* infection, parasite eggs are trapped in host liver and induce granulomatous inflammation, leading to liver fibrosis and severe damage. The mechanisms by which hepatic homeostasis is maintained are of central importance to inflammatory diseases.¹⁷ However, little is known about whether tissue-specific factor contributes to the regulation of granulomatous inflammation during schistosome infection. Here, for the first time, we have uncovered a novel role for hepatocytes of non-haematopoietic origin in protecting against hepatic damage in mice with schistosomiasis japonica.

Hepatocytes, which constitute approximately 60% of the liver by cell number and 80% by mass, are critical for maintaining hepatic homeostasis during inflammation.¹⁷ NKT cell subset is one of the important mediators of hepatic inflammation,²⁷ and CD1d constitutively expressed by hepatocyte has been reported to control local NKT cell numbers and maintain homeostasis in the liver.¹⁴ Surprisingly, we initially observed a dramatic decrease in hepatocellular CD1d expression in *S. japonicum*-infected mice, even starting before parasite egg laying. Of note, the dramatic decrease is mainly limited to hepatocytes of non-haematopoietic origin, but not DCs, macrophages, CD3⁺ T cells or B cells of haematopoietic origin, which show no change or just a moderate decrease in CD1d expression after *S. japonicum* infection. Based on the above, our hypothesis is that loss of hepatocellular CD1d expression upon *S. japonicum* infection may contribute substantially to the development of granulomatous inflammation in liver.

AAV8 yields the highest transduction rate of murine hepatocytes.²⁴ Using AAV8-mediated gene transfer to overexpress CD1d in hepatocytes, we show that CD1d is involved in protection against pathological damage resulted from *S. japonicum* infection. Coincidentally, study reported that a similar phenomenon has also been observed in inflammatory bowel disease, showing that CD1d on intestinal epithelial cells of non-haematopoietic cell origin is critical for maintaining mucosal homeostasis and a defect of epithelial CD1d expression parallels to intestinal inflammation.²⁸ Taken together, our study puts forward an important notion that CD1d harbours cell type-specific roles and parenchymal cells (non-haematopoietic cell origin) play an important role in protecting tissue from inflammation and sustaining local homeostasis through CD1d, in contrast to CD1d on cells

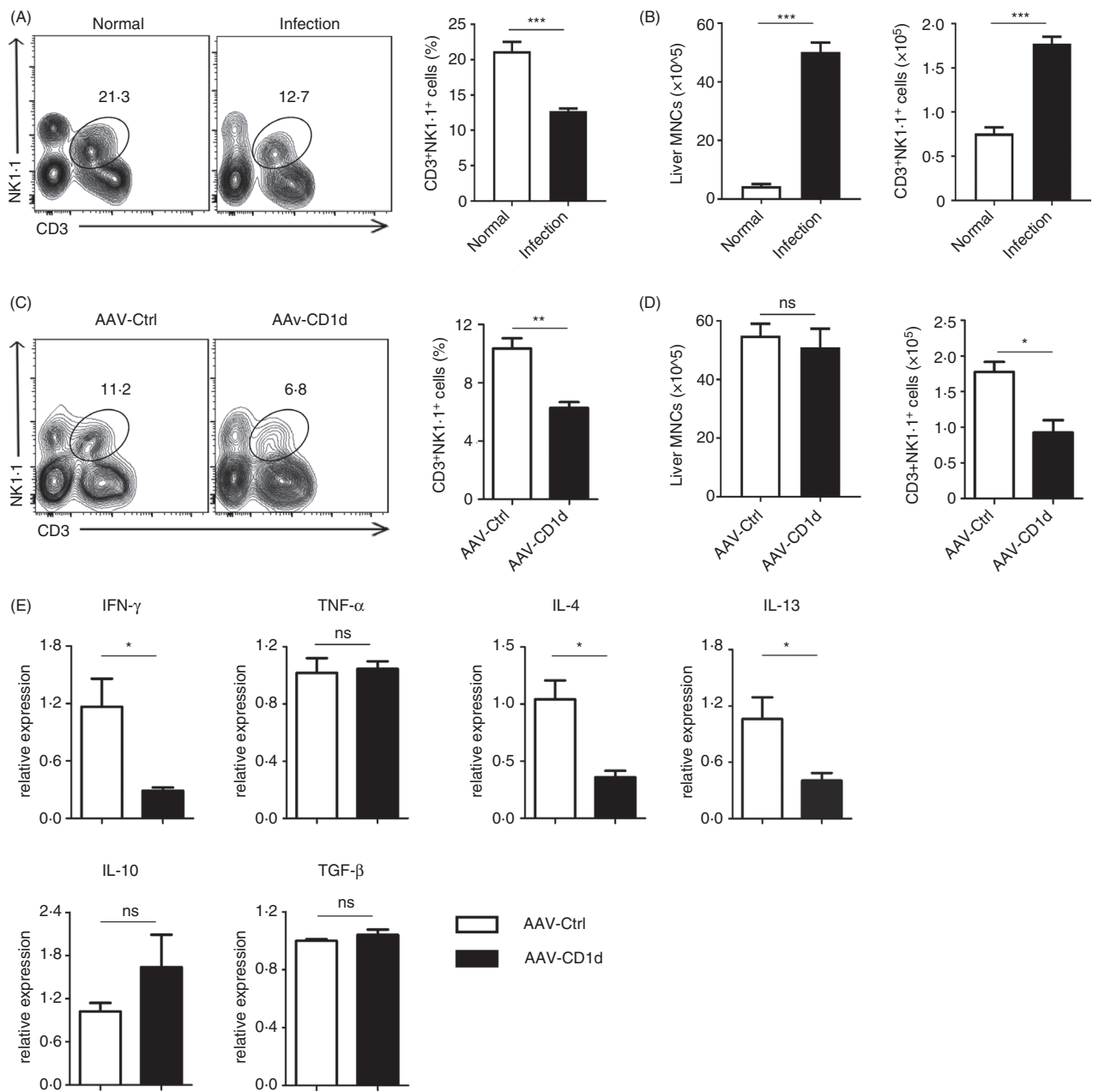


Figure 4. NKT cells are decreased in CD1d-overexpressing liver of *S. japonicum*-infected mice. (A) Representative FCM dot plots and average percentages of CD3⁺NK1.1⁺ NKT cells from the liver of normal or *S. japonicum*-infected mice. (B) The bar graphs show the absolute numbers of liver MNCs (left) and CD3⁺NK1.1⁺ NKT cells (right) in the liver from mice in (A). (C) Representative FCM dot plots and average percentages of CD3⁺NK1.1⁺ NKT cells from the liver of *S. japonicum*-infected mice injected with AAV-CD1d or AAV-Ctrl. (D) The bar graphs show the absolute numbers of liver MNCs (left) and CD3⁺NK1.1⁺ NKT cells (right) in the liver from mice in (C). (E) Relative mRNA expression levels of IFN-γ, TNF-α, IL-4, IL-13, IL-10 and TGF-β in livers from *S. japonicum*-infected mice injected with AAV-CD1d or AAV-Ctrl. Data are means ±SD from 5 mice per group and representative of two independent experiments. **P* < 0.05, ***P* < 0.01 and ****P* < 0.001; ns, not significant.

of haematopoietic origin (DCs, macrophages, etc.), which mainly presents phagocytosed dead cell- or pathogen-derived lipid antigens to NKT cells and contributes to the pathogenesis of inflammatory diseases.

Previous studies suggest that the balance between stimulatory and inhibitory lipid antigens act as a rheostat in

the regulation of NKT cell-dependent immunity.^{29–31} Hepatocyte metabolism-derived self-lipid antigens are presented by CD1d and important for maintaining local NKT homeostasis.¹⁴ However, our results show that schistosome infection disrupts the balanced crosstalk between metabolism and immunity by reducing hepatocellular

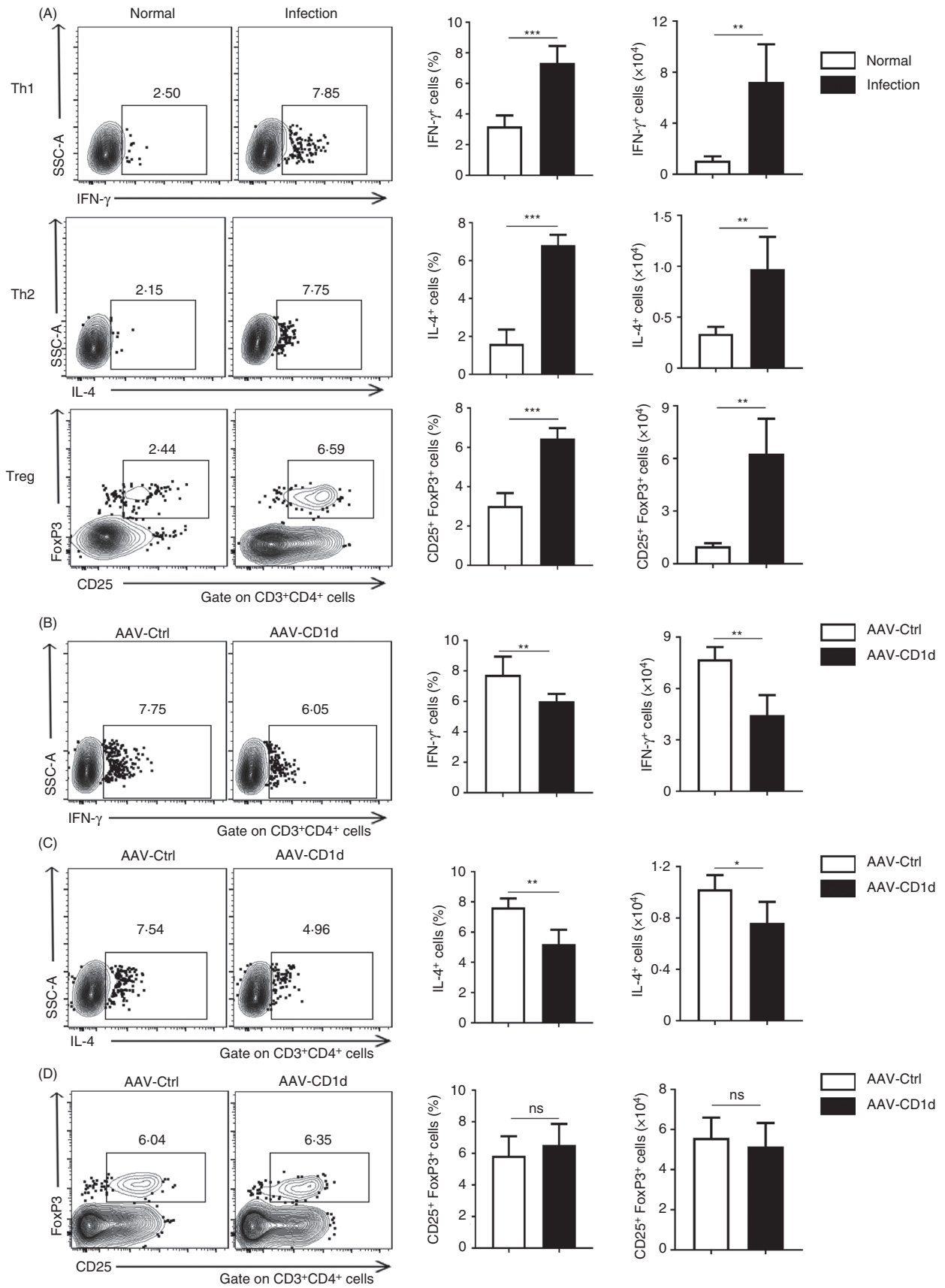


Figure 5. Both Th1 and Th2 cell responses are impaired in *S. japonicum*-infected mice with CD1d overexpression. (A) Representative FCM dot plots (left), average percentages (middle) and absolute numbers (right) for IFN- γ -(Th1)- and IL-4-(Th2)-producing CD3⁺CD4⁺ T cells, and CD3⁺CD4⁺CD25⁺Foxp3⁺ Treg cells from the livers of normal or *S. japonicum*-infected mice. (B–D) Representative FCM dot plots (left), average percentages (middle) and absolute numbers (right) for IFN- γ -(Th1)- and IL-4-(Th2)-producing CD3⁺CD4⁺ T cells (B and C), and CD3⁺CD4⁺CD25⁺Foxp3⁺ Treg cells (D) from the livers of *S. japonicum*-infected mice injected with AAV-CD1d or AAV-Ctrl. Data are means \pm SD from 5 mice per group and representative of two independent experiments. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$; ns, not significant.

CD1d. As such, our study indicates that CD1d may be a promising therapeutic target to rescue hepatocyte-mediated protection against liver damage in schistosomiasis.

Professional antigen-presenting cells (APCs), notably dendritic cells, present glycolipid antigens to NKT cells by CD1d during schistosome infection, leading to the activation and expansion of NKT cells and therefore the induction of both Th1 and Th2 responses and to egg-induced hepatic pathology.^{11,12,32} Hepatocellular CD1d is reported to control local NKT cell numbers by inducing their apoptosis and therefore maintain immunological homeostasis in the liver.¹⁴ Consistently, we observed that overexpression of hepatocellular CD1d in *S. japonicum*-infected mice induced a significant decrease in absolute number of hepatic NKT cells, thereby leading to corresponding decrease in both Th1 and Th2 responses, probably due to the reduced facilitation from NKT cells.^{7,25} All these observations suggest that CD1d plays variety, probably cell type-based roles during schistosomiasis. However, it should be noted that CD1d knockout mice with schistosomiasis have a reduced Th2 response and are protected from egg-induced hepatic pathology.¹¹ This could be explained by the fact that CD1d knockout mice without CD1d-expressing APCs have no functional NKT cells.^{33,34} Notably, epithelial CD1d uses a distinct mechanism to control intestinal inflammation, in a manner of inducing IL-10 production.³⁵ Interestingly, our data also showed an increase in IL-10 mRNA in CD1d-overexpressed liver, even though it was not statistically significant. Hepatic CD1d expression in *S. japonicum*-infected mice began to decrease as early as 1 week after infection. *S. japonicum* schistosomula arrive in the portal vein close to the liver by 3.5 ~ 6 days post-infection.^{36,37} Thus, the possible mediators causing the downregulation of hepatocyte CD1d in *S. japonicum* infection already exist from the very beginning of schistosomula stage. Additionally, in the present study we observed that several factors (SP1, PU.1 and LEF-1) in hepatocytes related to CD1d transcriptional regulation changed accordingly during *S. japonicum* infection, suggesting that multiple underlying mechanisms may be involved in the dramatic decline in hepatocellular CD1d expression. However, the exact reason why hepatocellular CD1d decreases so dramatically after *S. japonicum*-infected remains a mystery. Given the importance of hepatocellular CD1d in liver protection and its potential to be a tool for inflammation control, further research is needed in the future.

Taken together, our findings underscore the role of hepatocyte as a critical regulator in liver tissue inflammation and highlight the potential that hepatocellular CD1d defines a promising pathway to dampen hepatic inflammatory response during *S. japonicum* infection.

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DISCLOSURES

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

Data used for the analysis are available from the corresponding author upon reasonable request.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. IL-33 expression in hepatocyte from the liver of *S. japonicum*-infected mice.

Figure S2. SP1, PU.1, and LEF-1 mRNA levels in hepatocytes from *S. japonicum*-infected mice.

Figure S3. AAV8-mediated overexpression of CD1d does not affect NKT cells in the spleen of *S. japonicum*-infected mice.