Expanded Circulating Follicular Dendritic Cells Facilitate Immune Responses in Chronic HBV Infection

Xiaoyi Li
State Key Laboratory of Organ Failure Research, Guangdong Provincial Key Laboratory of Viral Hepatitis Research, Department of Infectious Diseases, Nanfang Hospital, Southern Medical University, Guangzhou, China https://orcid.org/0000-0003-0653-9587

Qifan Zhang
Division of Hepatobiliopancreatic Surgery, Department of General Surgery, Nanfang Hospital, Southern Medical University, Guangzhou, China

Wanyue Zhang
The Air Force Hospital of Southern Theater Command, Guangzhou, China

Guofu Ye
State Key Laboratory of Organ Failure Research, Guangdong Provincial Key Laboratory of Viral Hepatitis Research, Department of Infectious Diseases, Nanfang Hospital, Southern Medical University, Guangzhou, China

Yanchen Ma
State Key Laboratory of Organ Failure Research, Guangdong Provincial Key Laboratory of Viral Hepatitis Research, Department of Infectious Diseases, Nanfang Hospital, Southern Medical University, Guangzhou, China

Chunhua Wen
State Key Laboratory of Organ Failure Research, Guangdong Provincial Key Laboratory of Viral Hepatitis Research, Department of Infectious Diseases, Nanfang Hospital, Southern Medical University, Guangzhou, China

Shuqin Gu
State Key Laboratory of Organ Failure Research, Guangdong Provincial Key Laboratory of Viral Hepatitis Research, Department of Infectious Diseases, Nanfang Hospital, Southern Medical University, Guangzhou, China

Libo Tang
State Key Laboratory of Organ Failure Research, Guangdong Provincial Key Laboratory of Viral Hepatitis Research, Department of Infectious Diseases, Nanfang Hospital, Southern Medical University, Guangzhou, China

Yongyin Li (yongyinli@foxmail.com)

State Key Laboratory of Organ Failure Research, Guangdong Provincial Key Laboratory of Viral Hepatitis Research, Department of Infectious Diseases, Nanfang Hospital, Southern Medical University, Guangzhou, China https://orcid.org/0000-0001-6303-7642

Research

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Abstract

Background: Restoration of host hepatitis B virus (HBV)-specific antiviral immunity is an effective strategy for hepatitis B recovery. Follicular dendritic cells (FDCs) play a crucial role in immune regulation. This study aims to investigate the characteristics and functions of FDCs in chronic HBV infection.

Methods: The frequencies of FDCs in peripheral blood, liver, and spleen were measured in patients with chronic HBV infection. Isolated FDCs from splenic tissues of HBV-related liver cirrhosis-induced hypersplenism patients were cultured with autologous intrasplenic $CD4^+$ T cells and $CD19^+$ B cells.

Results: We found that patients with chronic HBV infection had a significantly increased frequency of circulating FDCs compared with that of healthy controls. Additionally, the frequency of circulating FDCs was positively correlated with that of intrahepatic and intrasplenic counterparts. Moreover, a positive correlation between the frequency of circulating FDCs and plasmablast and memory B cells, as well as C-X-C motif chemokine receptor type 5 $(CXCR5)^+CD4^+$ T cells and $CXCR5^+CD8^+$ T cells was also observed. Notably, *in vitro* experiments demonstrated that FDCs derived from splenic tissues of chronic HBV patients facilitated interferon-γ and interleukin-21 production from autologous intrasplenic $CD4^+$ T cells and promoted the proliferation of autologous intrasplenic $CD19^+$ B cells.

Conclusions: Expanded FDCs in patients with chronic HBV infection may favor the host immune responses against HBV. The identification of this unique population may contribute to a better understanding of the immune regulatory mechanisms and provide a potential immunotherapeutic target in chronic HBV infection.

Background

Chronic hepatitis B virus (HBV) infection is a major global health burden and may lead to progressive liver diseases such as chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC). As current antiviral therapies mainly inhibit HBV DNA replication by targeting the process of reverse transcription and have no direct effects on covalently closed circular DNA (cccDNA), chronic hepatitis B (CHB) patients tend to relapse after drug withdrawal [1–4]. Therefore, restoration of antiviral immunity against HBV may contribute to persistent viral suppression and even achieve durable viral clearance. Antibody against hepatitis B surface antigen (HBsAb) can neutralize circulating hepatitis B surface antigen (HBsAg) and clear HBV particles in vivo [5, 6]; however, the vast majority of patients with chronic HBV infection failed to generate HBsAb, even in patients with the achievement of HBV clearance, suggesting a profoundly compromised humoral immunity in chronic HBV patients. Hence, restoring HBV-specific B cell immunity may be an effective strategy for the cure of hepatitis B.

Germinal center (GC) is a specialized microstructure that forms in secondary lymphoid tissues following immunization. GC immune response is a critical process in the host-specific immune response. Notably, the immune response of B cells predominantly depends on the GC structure, including clonal expansion,

somatic hypermutation, affinity-based selection, and differentiation into plasma cells to produce protective high-affinity antibodies [7, 8]. The initiation and maintenance of GC require the collaboration of multiple leukocytes, including B cells, follicular helper T (Tfh) cells, follicular regulatory T (Tfr) cells, follicular cytotoxic T (Tfc) cells, and follicular dendritic cells (FDCs) [9]. In a previous study, we have demonstrated that C-X-C motif chemokine receptor type 5 $(CXCR5)^+$ $CD4^+$ T cells promote the proliferation of B cells and boost the production of HBV-related antibodies through the mediator interleukin (IL)-21 [10]. We have also found $CXCR5^+CD8^+$ T cells, a unique population that differs from the traditional $CD8^+$ T cells, possess more potent abilities to suppress HBV and facilitate B cells to produce HBV-specific antibodies by secreting IL-21 [10]. However, the characteristics and functions of FDCs in patients with chronic HBV infection remain largely unknown.

A previous study has documented that FDCs emerge from perivascular precursors [12], which is morphologically similar to DCs but displays distinct phenotypes and functions [13]. Generally speaking, FDCs were characterized with $CD14^+$, $CD21^{high}$, and FDC^+ (cloned CNA.42), while B cells ($CD21^+CD14^-$), monocytes ($CD21^-CD14^+$) and macrophages (FDC^-) are excluded in the case of such markers [14, 15]. FDCs retain native antigens in the form of immune complexes (ICs) for prolonged duration and present antigens to B cells during the secondary response. Additionally, FDCs are critical for rescue and activation of B cells by secreting IL-6 and B cell-activating factor (BAFF) [15–17]. However, as FDCs are fragile and tightly associated with B cells, it is challenging to perform FDCs isolation and investigate their functions *in vitro* [15]. In this study, we preliminarily dissected the characteristics and functions of FDCs in chronic HBV infection.

Methods

Patients and samples

Thirty-one treatment-naïve patients with chronic HBV infection were recruited and classified into immune tolerant carrier (IT; n = 13), hepatitis B e antigen (HBeAg)-positive CHB (n = 9), and inactive carrier (IC; n = 9) groups according to American Association for the Study of Liver Diseases guidelines [18]. Ten healthy controls (HCs) were enrolled. We collected matched peripheral blood mononuclear cells (PBMCs) and intrahepatic mononuclear cells (HMCs) from 11 treatment-naïve patients with HBV-related HCC who underwent curative hepatectomy. In addition, matched PBMCs and intrasplenic mononuclear cells (SMCs) were obtained from another 11 patients who underwent splenectomy due to HBV-related liver cirrhosis-induced hypersplenism. All individuals were recruited at Nanfang Hospital (Guangzhou, China). Exclusion criteria for these studies were coinfection with hepatitis A virus, hepatitis C virus, hepatitis D virus, hepatitis E virus, and human immunodeficiency virus (HIV). Patients with primary biliary cirrhosis and autoimmune diseases were also excluded. All individuals provided written informed consent, and the studies were approved by the Ethical Committee of Nanfang Hospital.

Mononuclear cells isolation

Twenty milliliters of heparinized blood was collected from patients with chronic HBV infection and HCs. The preparation of human HMCs was performed according to a previously described procedure [19]. Briefly, the liver tissues were flushed using 4°C RPMI-1640 complete medium (Gibco; Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 2% heat-inactivated fetal bovine serum (FBS; Hyclone; GE Healthcare Life Sciences, Logan, UT, USA), 100 μg/mL streptomycin, 100 U/mL penicillin, and 2mM ethylenediaminetetraacetic acid (EDTA; Ambion; Applied Biosystems, San Mateo, CA, USA). Subsequently, PBMCs and HMCs were isolated by density-gradient centrifugation on Ficoll-Hypaque and were cryopreserved in liquid nitrogen. Human SMCs were isolated as previously described [11, 19]. Briefly, the splenic tissues (2 cm^3) were placed into a 70 μm nylon mesh filter in a culture dish with the medium, and then the rounded side of a plunger from a 10 mL syringe was used to crush the tissues mechanically. The generated single-cell suspensions were used to obtain SMCs by Ficoll-Hypaque centrifugation. Cell viability, as assessed by trypan blue exclusion, was always higher than 90%.

Flow cytometry

PBMCs, HMCs, and SMCs were stained with the following monoclonal antibodies (mAbs) for 30 min at 4°C: CD14-PECy7, CD21-APC, FDC-FITC (cloned CNA.42), CD3-FITC, CD4-PECy7, CD8-APC, CXCR5-Brilliant Violet™ 421, CD19-APC, CD10-PE, CD38-FITC, and CD27-PerCPCy5.5. Dead cells were excluded using Live/Dead (Thermo Fisher Scientific; Waltham, MA, USA), cells were incubated with human BD Fc Block (5μL/million cell in 100 μL FACS buffer) for 10 min at room temperature to block the Fc-receptors. All samples were analyzed on a BD FACS CantoII flow cytometer (BD Biosciences). The data were analyzed with FlowJo software (Tree Star).

Enzyme-linked immunosorbent assay (ELISA)

The plasma levels of IL-6, IL-7, IL-15, BAFF (Invitrogen; Carlsbad, CA, USA), and C-X-C motif chemokine ligand 13 (CXCL13; R&D Systems; Minneapolis, MN, USA) and the concentrations of IL-6, IL-21, interferon (IFN)-γ (Invitrogen; Carlsbad, CA, USA), and CXCL13 in the culture supernatants were assessed by commercially available ELISA kits according to the manufacturers' instructions.

Isolation and culture of FDCs

Primary human FDCs were established as described previously [15]. Briefly, human splenic tissues were placed in petri dishes and washed several times with PBS, followed by cut into small pieces. The spleen fragments were then digested with 0.5mM EDTA and 0.25% of trypsin for 20 min at 37°C and stopped the reaction with cold RPMI-1640 complete medium supplemented with 10% heat-inactivated FBS. The suspension was filtered through gauze and centrifuged. The supernatant was discarded, and the cell pellet was suspended in the medium and centrifuged on Ficoll-Hypaque. The interlayer cells were collected and incubated in culture flasks for 1 h at 37°C in RPMI-1640 complete medium with 10% heat-inactivated FBS to allow the adherence of macrophages and granulocytes. The supernatant cells were washed and incubated in a fibroblast medium, which consists of Opti-MEM reduced serum medium (Gibco; Thermo Fisher Scientific; Waltham, MA, USA) and a supplement containing 3% FBS, 100 U/mL

penicillin, 50 μg/mL gentamicin, and 1 mmol/L glutamine. After overnight incubation, adherent cells attached to the flask, lymphocytes in the supernatant were discarded. The fibroblast medium was replaced and changed twice a week. FDCs matured after 2–4 weeks of culture.

Immunofluorescence staining and confocal microscopy

Cultured FDCs were transferred into the confocal petri dishes and washed with PBS and fixed in 4% paraformaldehyde for 10 min at room temperature. The cells were then treated with 0.5% Triton X-100 for 5 min and blocked with 1% bovine serum albumin (BSA; Fdbio Science; Hangzhou, China) for 30 min at room temperature. Subsequently, the cells were stained with mouse anti-human FDC monoclonal antibody (CNA.42, 1:300, eBioscience) overnight at 4°C. Following three washes with PBS, the cells were incubated with the goat anti-mouse antibody (1:400, Jackson ImmunoResearch) for 1 h at room temperature. Then confocal petri dishes were stained with DAPI (Abcam). Images were acquired using a confocal laser scanning microscope (Fluoview FV10i; Olympus, Tokyo, Japan).

Cell culture and supernatant analysis

Cultured FDCs were stimulated with IL-4, IL-10, IL-21, lipopolysaccharide (LPS), IFN-α-2α, lymphotoxin (LT)-α1β2, tumor necrosis factor (TNF)-α, CPG, and PMA for 3 days, respectively. The supernatants were collected and the levels of IL-6 and CXCL13 were assessed by ELISA. Autologous $CD4^+$ T cells and $CD19^+$ B cells were sorted from SMCs of patients with chronic HBV infection by FACS and cryopreserved in liquid nitrogen. Purified autologous $CD4^+$ T cells were thawed and plated in a 96-well plate that pre-seeded with FDCs at an 80:1 ($CD4^+$ T cells/FDCs) ratio, or with medium only as a control, and co-cultured in the presence of IL-2 (10ng/mL) and anti-CD3/CD28 (10μg/mL) for 3 days, the supernatants were collected and the levels of IL-21 and IFN-γ were assessed by ELISA.

Proliferation assay

Purified intrasplenic $CD19^+$ B cells were thawed and labeled with carboxyfluorescein succinimidyl ester (CFSE; 1.5 mM; Molecular Probes; Eugene, OR) and suspended at 10^6 cells/mL. Labeled cells were plated in a 96-well plate that pre-seeded with FDCs at an 80:1 ($CD19^+$ B cells/FDCs) ratio, or with medium only as a control, and co-cultured in the presence of CPG (10μg/mL) for 7 days. The proliferation rate of B cells was expressed as the percentage of cells that diluted CFSE intensity at least once at the time of harvest.

Statistical analysis

Data are expressed as either the median (range) or the mean ± SD. Statistical analyses were performed in GraphPad Prism v.8.0.1 (La Jolla, CA, USA). Mann-Whitney U test was used when two groups were compared. Correlations between variables were assessed with the Spearman rank-order correlation coefficient. All statistical analyses were based on two-tailed hypothesis tests with a significance level of $p < 0.05$.

Results

A positive correlation between the frequency of circulating FDCs and intrahepatic and intrasplenic FDCs was found in chronically HBV-infected patients.

FDCs are mainly located in lymphoid tissue; however, it is difficult to obtain enough lymphoid tissues to investigate the role of FDCs in chronic HBV infection. According to a previous study [14], we quantified the frequencies of FDCs with the indicated gating strategy (Fig. 1a). Firstly, we aim to find out the relationship between intrasplenic and circulating FDCs. Not surprisingly, the frequency of intrasplenic FDCs was significantly higher than that of circulating FDCs, and a positive correlation between the frequency of intrasplenic FDCs and circulating FDCs was found (Fig. 1b and 1c). As Hepatitis B virus (HBV) is a prototype member of the hepadnaviridae, we then analyzed intrahepatic FDCs. Similar to the findings of intrasplenic counterparts, the frequency of intrahepatic FDCs was significantly increased, and a positive correlation between the frequency of intrahepatic FDCs and circulating FDCs was also observed (Fig. 1b and 1c). Collectively, these findings suggest that analysis of circulating FDCs may serve as an optional surrogate for the assessment of lymphoid FDCs in chronic HBV infection.

Circulating FDCs are expanded in patients with chronic HBV infection.

To find out whether chronic HBV infection could drive FDCs expansion, we measured the frequencies of circulating FDCs in $CD14^+$ cells in patients with chronic HBV infection and HCs. As shown in Fig. 2a, a significantly higher frequency of FDCs was observed in patients with chronic HBV infection compared with the HCs group. Notably, compared to that in IT patients, CHB patients and IC patients had a significantly increased percentage of FDCs (Fig. 2b). It is known that the serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), HBsAg, HBeAg, antibody against hepatitis B e antigen (HBeAb), and HBV DNA are clinical indicators of HBV infection. We then examined the relationships between the frequency of circulating FDCs and the levels of viral and biochemical parameters. However, we found the serum levels of ALT, AST, HBsAg, HBeAg, HBeAb, and HBV DNA were not correlated with the frequency of circulating FDCs (Fig. 2c-f). Overall, these data indicate that chronic HBV infection may induce the expansion of circulating FDCs.

A positive correlation between the frequency of circulating FDCs and immune cell subsets in patients with chronic HBV infection

Since FDCs promote B cell proliferation and differentiation by secreting cytokines, such as IL-6, IL-7, IL-15, BAFF, and CXCL13 [17, 20], we then investigated the correlation between the frequency of circulating FDCs and B cell subsets in patients with chronic HBV infection. Interestingly, we found that the frequency of circulating FDCs was positively correlated with plasmablast and memory B cells. In contrast, the frequency of circulating FDCs was inversely correlated with the frequency of circulating naïve B cells, while no significant correlation existed between the frequency of circulating FDCs and immature B cells (Fig. 3a). We also investigated the relationship between intrasplenic FDCs and intrasplenic B cell subsets. However, there was no significant correlation existed between the frequency of intrasplenic FDCs and

intrasplenic B cell subsets (Additional file 1: Figure S1). Moreover, as $CXCR5^+CD4^+$ T cells and $CXCR5^+CD8^+$ T cells are critical for GC immune responses [10, 11], we further investigated the frequencies of these two subpopulations in peripheral blood and spleen. Importantly, we found that the frequency of circulating FDCs was positively correlated with the frequencies of $CXCR5^+CD4^+$ T cells and $CXCR5^+CD8^+$ T cells (Fig. 3b). Correspondingly, similar findings were also found between intrasplenic FDCs and $CXCR5^+CD4^+$ T cells and $CXCR5^+CD8^+$ T cells (Additional file 2: Figure S2). Additionally, we assessed the plasma levels of IL-6, IL-7, IL-15, BAFF, and CXCL13 in patients with chronic HBV infection by ELISA. However, no significant correlation was found between the frequency of circulating FDCs and plasma levels of the aforementioned cytokines (Fig. 3c). Collectively, these results suggest that FDCs are expanded in chronic HBV infection and may influence B cell differentiation and GC immune responses.

FDCs facilitate cytokines production from $CD4^+$ T cells and the proliferation of B cells.

To investigate the role of FDCs *in vitro*, FDCs from splenic tissues were isolated from patients who underwent splenectomy due to HBV-related liver cirrhosis-induced hypersplenism and incubated in the fibroblast medium. After 25 days, the FDCs network structure was formed and could be observed with an optical microscope (Fig. 4a). The FDCs were then labeled with an anti-FDC fluorescent dye and were further verified by confocal microscopy (Fig. 4b). To investigate the ability of cytokines production from FDCs *in vitro*, cultured FDCs were stimulated with different reagents (IL-4, IL-10, IL-21, LPS, IFN-α-2α, LT-α1β2, TNF-α, CpG, or PMA). As shown in Fig. 4c, an increased level of IL-6 was observed under the stimulation of IL-4, as well as in the context of IL-10, LPS, IFN-α-2α, and TNF-α stimulation. Intriguingly, the production of chemokine CXCL13 from FDCs was solely found under the stimulation of LT-α1β2 (Fig. 4d). Next, we further determined the effects of FDCs on T cells and B cells. When cultured FDCs were co-cultured with autogenous intrasplenic $CD4^+$ T cells, we found elevated expression of IL-21 and IFN-γ from the supernatant (Fig. 4e and 4f). Also, an increase in cell proliferation was found in the co-culture condition of cultured FDCs and autogenous intrasplenic $CD19^+$ B cells (Fig. 4g). In summary, these findings indicated that FDCs might facilitate cytokine production from $CD4^+$ T cells and promote B cell proliferation.

Discussion

Humoral immune responses play an essential role in the control of viral infection. However, previous studies have documented a comprised HBsAg-specific B cell response in patients with chronic HBV infection [10, 11], and the reversal of B-cell functional impairment is associated with HBsAg seroconversion [23]. Of note, it has been reported that FDCs are essential for B cell survival and motivate the process of affinity maturation during GC reaction [24, 25]. The definition of mice FDCs with FDC-M1 has been generally accepted, while phenotypes of human FDCs remain controversial [26]. According to the report from a previous study, human FDCs were characterized by $CD14^+$ $CD21^{high}$ FDC^+ in the present study. Based on this definition, B cells ($CD21^+$ $CD14^-$), monocytes ($CD21^-$ $CD14^+$), and macrophages

(FDC$^-$) are excluded [14]; however, this definition is inadequate because of the tissue specificity of FDCs. Therefore, the FDCs described in this study should be defined more accurately as CD14$^+$ FDCs [27].

A previous study has demonstrated that FDCs are closely associated with the residency and proliferation of tumor cells in follicle-derived lymphomas, even when the infiltration localizes in the bone marrow and non-lymphoid organs [28]. Moreover, another study has demonstrated that the precursors of FDCs are perivascular cells and can develop into mature FDCs under certain conditions [12], which indicates that FDCs may not restrict in the lymphatic organ. However, FDCs have not been well investigated in peripheral blood. In this study, we found that FDCs were mainly distributed in the spleen and liver. Interestingly, we also found a small quantity of FDCs in peripheral blood in patients with chronic HBV infection. This phenomenon might be attributed to the directional migration of FDCs. In addition, inflammatory factors may induce the maturation of the FDCs precursors, and enhance the vascular permeability in chronic HBV infection, leading to the entry of mature FDCs to the blood. The difficulty for FDCs investigation lies in the fragile character and tightly association with B cells; therefore, an alternative method for assessment of FDCs in lymphoid organs is highly desired. We herein found a close correlation between circulating FDCs and intrahepatic and intrasplenic FDCs, suggesting that analysis of circulating FDCs may be an optional surrogate for the assessment of lymphoid FDCs in chronic HBV infection.

Recent studies have reported the critical role of FDCs in chronic infectious diseases. Importantly, FDCs are a critical HIV reservoir where FDCs-trapped HIV persists for long periods, resulting in the perpetuation and reignition of the disease [29–31]. FDCs also play an essential role in autoimmune disorders, which frequently display follicles with ICs-bearing FDCs, autoreactive GCs, and ongoing affinity maturation [32, 33]. However, the characteristics and functions of FDCs in HBV infection are poorly understood. In the present study, our results showed that a significantly higher frequency of circulating FDCs was observed in individuals with chronic HBV infection, compared with HCs group. However, there was no correlation between the frequency of circulating FDCs and clinical indicators in chronic HBV infection. To study the role of FDCs in the prognosis of HBV infection, we analyzed and found an increased frequency of circulating FDCs in patients with immune active phase (CHB) compared with that in IT patients, which indicated that immune activation-induced inflammatory milieu might promote the expansion of FDCs. Nevertheless, a high frequency of FDCs was also found in IC patients, which might be associated with the influence of HBV antigens. We speculated that the production of large amounts of antigens in patients with chronic HBV infection might inhibit the expansion of FDCs. In contrast, a low load of HBV antigens, just as observed in IC patients, may relieve the suppressed status of FDCs. Previous studies have reported that B cells are programmed to apoptosis in the light zone of GC. Only after positive selection by FDCs and Tfh cells, can B cells survive and return to the dark zone for proliferation cycle or differentiate into plasma cells and then leave the GC to participate in humoral immune response [34]. In this study, we showed that the frequency of circulating FDCs is positively correlated with plasmablast and memory B cells but negatively correlated with naïve B cells in patients with chronic HBV infection, suggesting a potential effect of FDCs on the humoral immune response. Furthermore, as a skeletal component of GC,

FDCs may play a critical role in regulating the differentiation and function of immune cells in GC, such as Tfh cells, Tfc cells, and B cells. Previous studies from our group have verified the role of $CXCR5^+CD4^+$ T cells, as well as $CXCR5^+CD8^+$ T cells on HBV control. In the present study, we found a positive association between the frequency of circulating FDCs and $CXCR5^+CD4^+$ T cells, as well as $CXCR5^+CD8^+$ T cells both in blood and spleen. In addition, a variety of cytokines that produced from FDCs, such as BAFF, IL-6, and IL-15 involve in the activation and follicular homing of B-cells [35–38]; however, there was no correlation between the frequency of circulating FDCs and plasma levels of cytokines in our analysis. This might attribute to the fact that FDCs exert effects mainly in the GC, and the plasma cytokine levels reflect the general condition of individuals.

Due to the rare quantity of FDCs, we established primary human FDCs for *in vitro* experiments. Notably, we carried out the experiments about the functions of FDCs after cultured FDCs were further verified by immunofluorescence under a confocal microscope in this study. It has been reported that FDCs produce high levels of IL-6, which is critical to proinflammatory and immune regulatory cascades [39]. Besides, FDCs also secret the chemokine CXCL13, which mediates the homing of B cells towards GC [17]. Our results indicated that FDCs could produce IL-6 and CXCL13 under stimulation *in vitro*. It was also found that FDCs promoted $CD4^+$ T cells cytokines production and B cell proliferation in the co-culture system, whether this effect is mediated by IL-6 or CXCL13 needs further investigation. FDCs have been reported to deliver antigens to B cells in the form of ICs [17]. Intriguingly, in the present study, we found that the frequency of circulating FDCs is positively correlated with plasmablast and memory B cells in patients with chronic HBV infection, and FDCs promote the proliferation of B cells *in vitro*. Whether FDCs can deliver HBV antigens to B cells in the form of HBsAg - HBsAb ICs and whether FDCs can restore the immune functions of HBV-specific B cells warrant further study.

It should be noted and acknowledged that the current study has limitations, especially the lack of in-depth functional research of FDCs; this deficiency may in part attribute to the fragile character and the sparse amount of FDCs in chronic HBV infection.

Conclusions

The current study preliminarily dissected the characteristics and functions of FDCs, and revealed an expansion of FDCs as well as its favorable effects on $CD4^+$ T cells cytokines production and B cell proliferation in patients with chronic HBV infection, suggesting the regulatory effects of FDCs on anti-HBV immune responses. This study might provide new insights for the further understanding of the immune regulatory mechanism of chronic HBV infection.

List Of Abbreviations

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BAFF, B cell-activating factor; BSA, bovine serum albumin; cccDNA, covalently closed circular DNA; CFSE, carboxyfluorescein succinimidyl ester; CHB, chronic hepatitis B; CXCL13, C-X-C motif chemokine ligand 13; CXCR5, C-X-C motif chemokine

receptor type 5; DCs, dendritic cells; EDTA, ethylenediaminetetraacetic acid; FBS, fetal bovine serum; FDCs, follicular dendritic cells; GC, germinal center; HBeAb, antibody against hepatitis B e antigen; HBeAg, hepatitis B e antigen; HBsAb, antibody against hepatitis B surface antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCs, healthy controls; HIV, human immunodeficiency virus; HMCs, intrahepatic mononuclear cells; IC, inactive carrier; ICs, immune complexes; IFN, interferon; IL, interleukin; IT, immune tolerant carrier; LPS, lipopolysaccharide; LT, lymphotoxin; mAbs, monoclonal antibodies; PBMCs, peripheral blood mononuclear cells; SMCs, splenic mononuclear cells; Tfc, follicular cytotoxic T cells; Tfh, follicular helper T cells; Tfr, follicular regulatory T cells; TNF, tumor necrosis factor.

Declarations

Ethics approval and consent to participate: All individuals provided written informed consent, and the studies were approved by the Ethical Committee of Nanfang Hospital.

Consent for publication: Not applicable.

Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

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Authors' contributions: YYL, QFZ, and XYL designed the study. XYL and WYZ performed the experiments and analyses. XYL, QFZ, GFY, YCM, CHW, and SQG collected samples and laboratory data. YYL, LBT, and XYL wrote the manuscript. YYL supervised the study. All authors read and approved the final manuscript.

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Authors' information:[1] State Key Laboratory of Organ Failure Research, Guangdong Provincial Key Laboratory of Viral Hepatitis Research, Department of Infectious Diseases, Nanfang Hospital, Southern Medical University, Guangzhou, China; [2] Division of Hepatobiliopancreatic Surgery, Department of General Surgery, Nanfang Hospital, Southern Medical University, Guangzhou, China; [3] The Air Force Hospital of Southern Theater Command, Guangzhou, China

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Figures

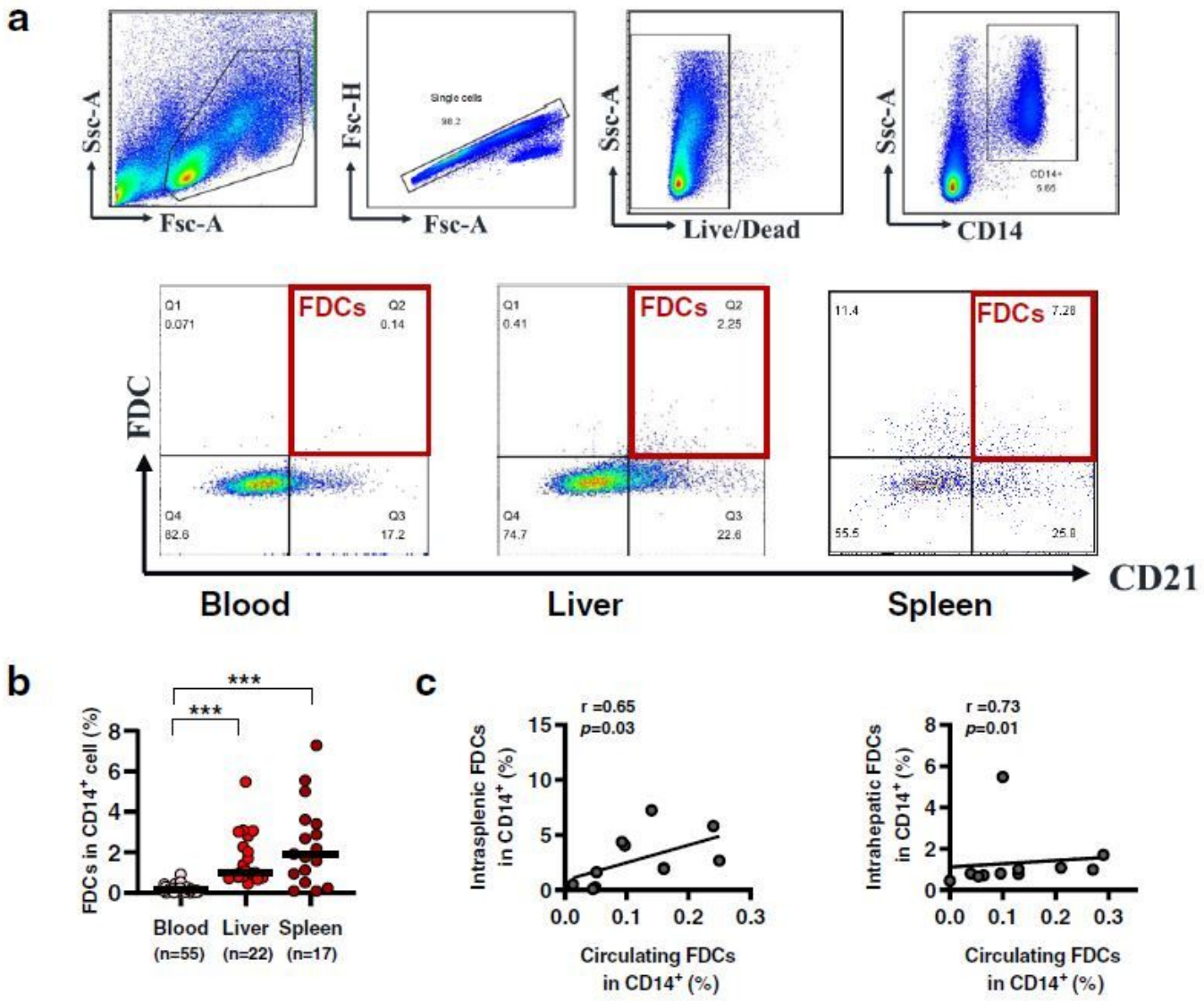

Figure 1

A positive correlation between the frequency of circulating FDCs and intrahepatic and intrasplenic FDCs. a Gating strategy for the identification of FDCs (CD14+CD21highFDC+) using flow cytometry. FDCs population was calculated as a percentage of CD14+ cells. b Comparison of the frequencies of circulating (n = 55), intrahepatic (n = 22) and intrasplenic (n = 17) FDCs in patients with chronic HBV infection. c The frequency of circulating FDCs was correlated with intrasplenic FDCs (left panel, n = 11) and intrahepatic FDCs (right panel, n = 11). ***P < 0.001. FSC-A, forward scatter; SSC-A, side scatter; Live/Dead, fixable dead cell stain.

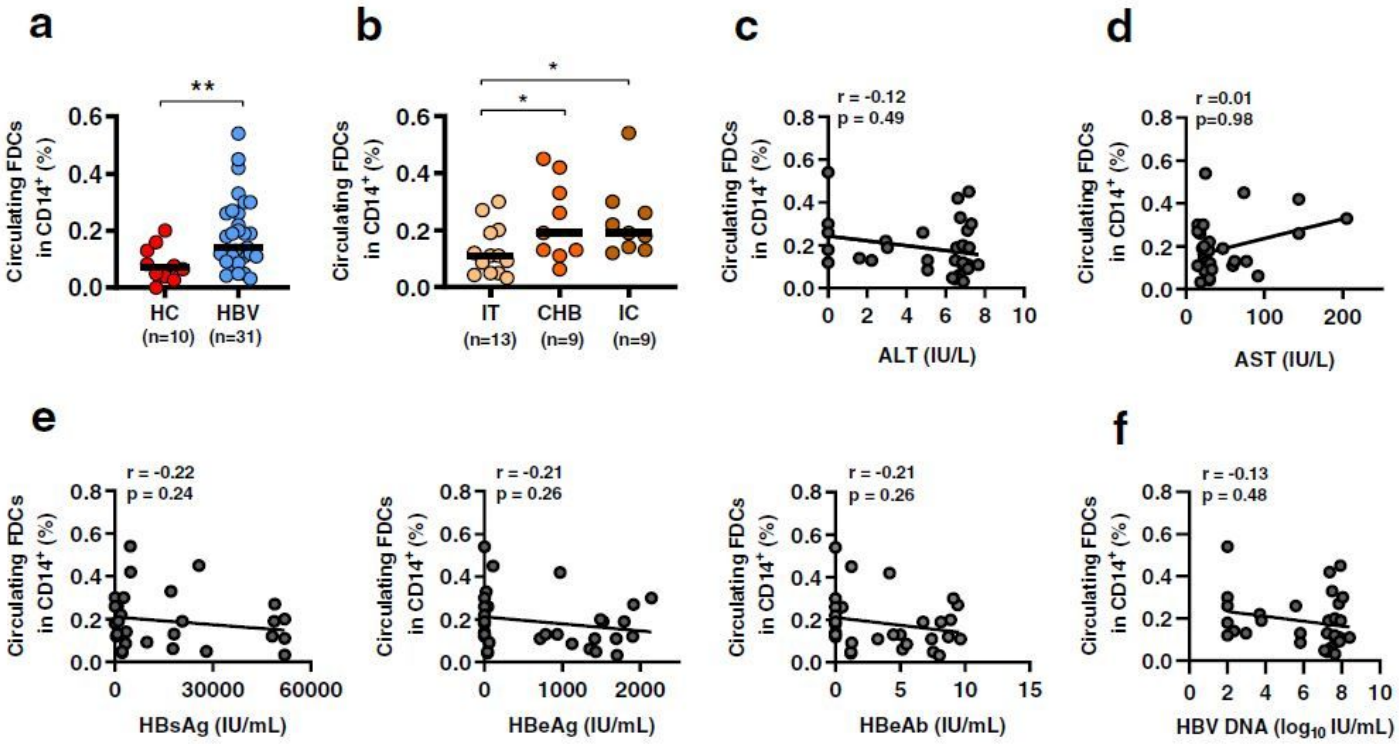

Figure 2

Circulating FDCs are expanded in patients with chronic HBV infection. a Comparison of the frequencies of FDC between healthy controls (HCs, n = 10) and patients with chronic HBV infection (HBV, n = 31). b Comparison of the frequencies of FDC between immune tolerant carrier (IT, n = 13), HBeAg-positive CHB (CHB, n = 9), and inactive carrier (IC, n = 9) patients. c-f Analyses of the correlations between the frequency of circulating FDCs and serum levels of ALT, AST, HBsAg, HBeAg, HBeAb and HBV DNA (n = 31). *P < 0.05, **P < 0.005.

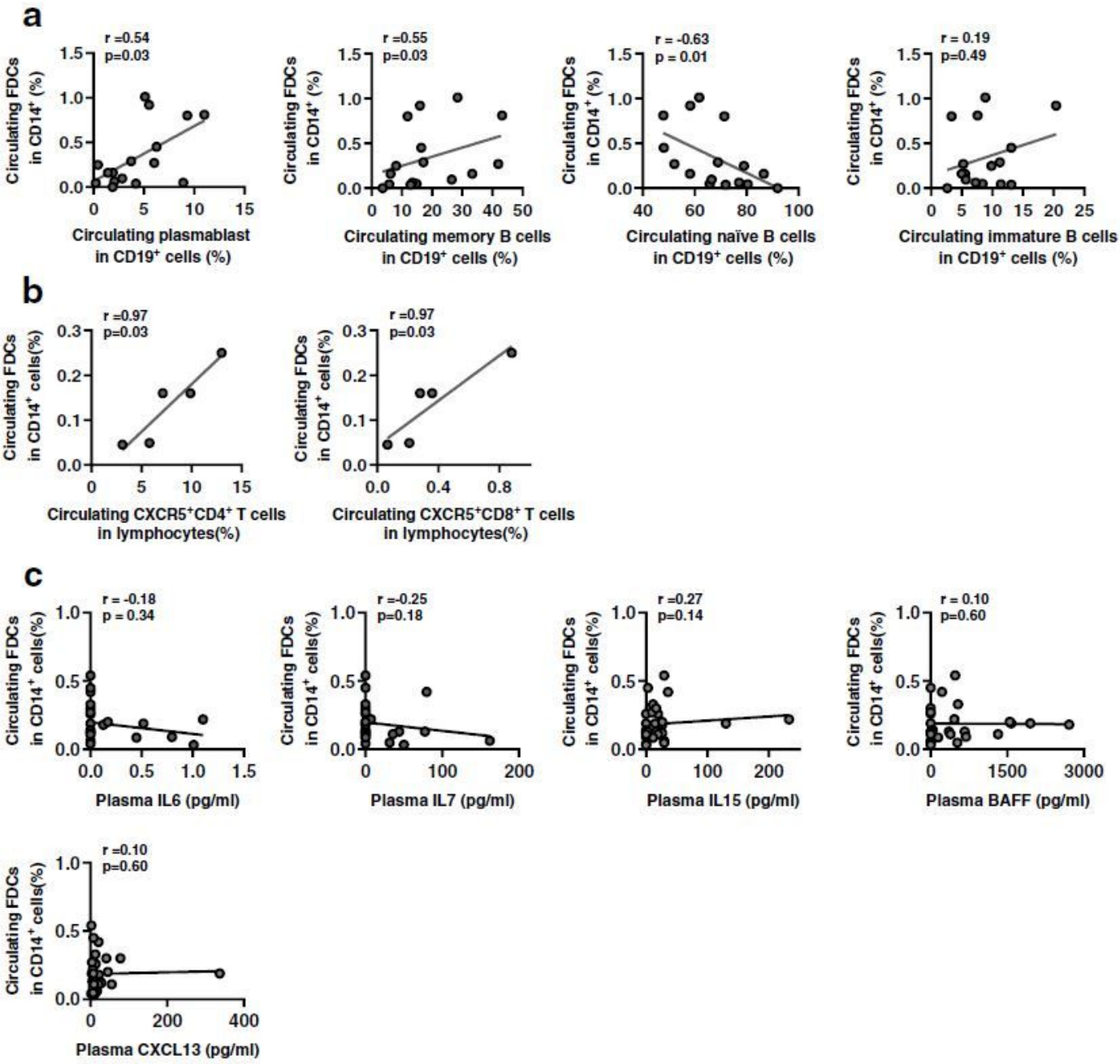

Figure 3

Correlations between the frequency of FDCs and immune cell subsets in chronic HBV-infected patients. a Correlations between the frequency of FDCs and plasmablast (CD19+CD38+CD27+), memory B cells (CD19+CD27+CD38-), naïve B cells (CD19+CD10-CD27-), and immature B cells (CD19+CD10+CD27-) in peripheral blood in chronic HBV patients (n = 16). b Correlations between the frequency of FDCs and CXCR5+CD4+ T cell and CXCR5+CD8+ T cell subsets in peripheral blood in chronic HBV patients (n = 5). c Correlations between the frequency of circulating FDCs and plasma levels of IL-6, IL-7, IL-15, BAFF, and CXCL13 in chronic HBV patients (n = 31).

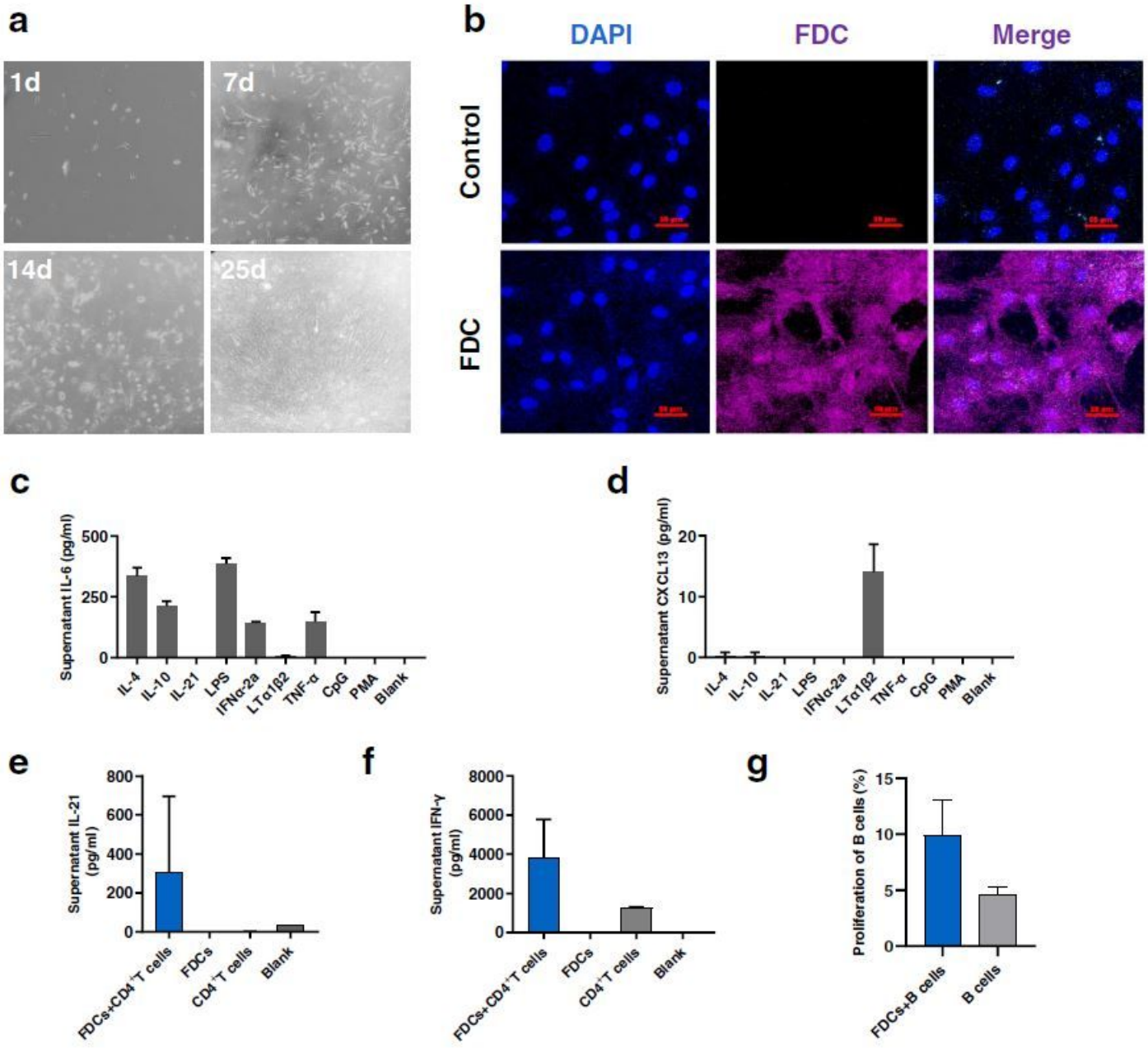

Figure 4

FDCs facilitate CD4+ T cells cytokines production and B cell proliferation in vitro. a Cultured FDCs were observed by optical microscopy at days 1, 7, 14, and 25, respectively. b Cultured FDCs were stained for FDC expression and analyzed by confocal microscopy, red for FDC and blue for DAPI. Photographs at 40× magnification. Cultured FDCs were stimulated with indicated reagents for 3 days and the supernatants were collected for IL-6 (c) and CXCL13 (d) detection by ELISA. Cultured FDCs were co-cultured with purified autologous CD4+ T cells in the presence of IL-2 (10ng/mL) and anti-CD3/CD28 (10μg/mL) for 3 days and the supernatant were collected for IL-21 (e) and IFN-γ (f) detection by ELISA. g Purified autologous CD19+ B cells were labeled with CFSE, then were co-cultured with cultured FDCs in the presence of CPG (10μg/mL) for 7 days and the proliferation of B cells was measured by flow cytometry.

Supplementary Files

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