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Decreased Frequency and Function of Circulating Plasmocytoid Dendritic Cells (pDC) in Hepatitis B Virus Infected Humans

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The Type 2 precusor plasmacytoid dendritic cells (pDC) represent the most important cell type in antiviral innate immunity. To understand the function of pDC during hepatitis B virus infection, the frequency and function of circulating pDC were analyzed by flow cytometric analysis, and IFN-α secretion of total PBMCs was determined by ELISA assay in 25 healthy subjects and 116 patients at various stages of chronic hepatitis B virus infection (CHB). The number of circulating pDC was found to be significantly lower in patients with CHB and associated liver cirrhosis (LC). The ability of PBMCs to secrete IFN-α also decreased significantly. There was a corresponding decrease of circulating NK cells and $CD8^+$ T cells. We observed that lamuvidine antiviral therapy restored the number of circulating pDC and there was a reversal of pDC frequency with the control of HBV replication in chronic HBV patients, indicating these subjects are unlikely to be totally immunecompromised. The decrease of pDC was found to be related to nosocomial infections in LC patients. Our results suggest that CHB patients probably have a quantitative and qualitative impairment of circulating pDC or NK cells, which may be associated with HBV persistent infection as well as the nosocomial infections that arise in LC patients.

KEY WORDS: chronic hepatitis; dendritic cells; liver cirrhosis; nosocomial infections.

INTRODUCTION

Chronic hepatitis B virus (HBV) infection affects the estimated 400 million individuals worldwide and becomes a major public health problem, due to its consequences of fulminant hepatic failure, liver cirrhosis (LC), and primary hepatocellular carcinoma (HCC) (1–3). One of the most affected areas in the world is mainland China where almost 130 million people are in the HBsAg carrier state, of whom 23 million individuals have the chronic active hepatitis (4). Previous investigations have demonstrated that patients with acute symptomatic HBV infection successfully clear the infection because of the development of vigorous polyclonal class I-restricted cytotoxic T lymphocyte (CTL) and class II-restricted $CD4^+$ T-helper responses to HBV antigens in peripheral blood; these T cells are believed to be crucial to antiviral resistance (1, 5–7). Moreover, an important finding in the field showed that Th1 cytokines like IFN-γ and TNF-α produced by the activated immune cells in the liver can purge viruses from infected cells in a noncytopathic manner (8). By contrast, HBV persistence *in vivo* has been considered to be due to an inability of the adaptive immune system to clear the virus efficiently (9). Because of this reason, probably, most published studies on the immunopathogenesis of HBV infection focused on the analysis of the components in adaptive immunity (10, 11). However, recent studies suggest that innate immunity also plays a critical role in antiviral immune responses during acute (6, 8, 12) and chronic HBV infection (13). Most importantly, it is considered that innate immunity not only provides an immediate response to viral infection, helping to clear virus during the initial period of viral infection, but innate immunity also shapes the nature of the adaptive immune response to viral infection (6, 14). The evidence from animal models and HBV-infected patients also suggests that a fully integrated innate and adaptive immune response is needed to achieve a resolution of HBV infection in humans (6, 8). The different components of the both innate and adaptive immune systems are so interconnected *in vivo* that the defects of one or more may lead to the establishment

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of chronicity of HBV infection as well as unfavorable outcome.

Phenotypic analysis has revealed the presence of at least two major distinct subsets of dendritic cells (DCs) in human peripheral blood, myeloid DC, or monocyte-derived DC (mDC) that express CD11c, CD11b, CD13, and CD33 and are primarily associated with antigen uptake and activation of naïve T cells, and plasmacytoid dendritic cells (pDC) that express CD123, CD4, and $BDCA_2$, but lack CD11c and myeloid lineage markers (15, 16). pDC, also known as interferon-producing cells (IPC), can produce up to 1000 times more IFN-α than any other cell type when challenged with certain inactivated viruses (17, 18), and therefore pDC likely serve an important role in innate immunity against viral infection. Several studies have recently shown that viruses, such as hepatitis B or C virus (HBV or HCV) (4, 19), human immunodeficiency virus-1 (HIV-1) (20), vaccinia virus (21) and herpes simplex virus (22) may reduce the antiviral immune response by inducing a functional deficiency of mDC (4). Beckebaum (23, 24) and Arima *et al*. (25) reported that mDC are susceptible to HBV, and the presence of intracellular HBV virons is associated with an impaired allostimulatory function in CHB patients. Ninomiya *et al*. reported that patients with HBV- or HCV-related primary HCC displayed a phenotype defect and functional deficiency of antigen-presenting DC (26, 27). In HIV-1-infected individuals, a progressive loss of functional circulating pDC has recently been demonstrated in association with opportunistic infections (28–30), supporting a role for these cells in protective responses. To date, the potential role of pDC is still unexplored in humans with chronic HBV infection. Therefore, the aim of the study was to investigate the frequency of blood pDC in chronically HBV-infected patients. The numbers of total peripheral NK cells, $CD4^+$, and $CD8^+$ T cells were also studied in parallel.

MATERIALS AND METHODS

Study Subjects

We recruited 116 patients of Chinese Han ethnic origin at various stages of HBV infection, including 9 acute hepatitis (AH), 80 chronic hepatitis (CHB), and 27 liver cirrhosis (LC), as well as 25 age- and sex-matched healthy volunteers (normal controls, NC). The study protocol was approved by the Ethics Committees of our unit, and written informed consent was obtained from each subject. The clinical parameters of the subjects are shown in Table I. Patients with concurrent HCV or HIV infection were excluded in this study. HBV serologic markers, including HBsAg, HBsAb, HBeAg, HBeAb, and HBcAb, as well as anti-HCV and anti-HIV antibodies (Abbott Laboratories, Chicago, IL), were measured using commercially available ELISA kits (Beijing Kewei Diagnostic Reagent Plant, Beijing, China). AH patients are defined as those who all displayed HBsAg seroconversion within 6 months after the initial HBV infection. The time of sample collection in AH patients was during the phase of active replication and clinical symptoms and without antiviral therapy. Eighty of the CHB patients were positive for serum HBsAg, HBeAg, and HBcAb markers, abnormal elevations of aminotransferase activity (115.8 ± 69.9 IU/mL), and viral load ranged from 2.63×10^4 to 6.56×10^8 copies/mL. The patients in the LC group were older than those in the groups of CHB and AH, and the number of circulating white blood count (WBC) and neutrophils in LC group were at the lower levels compared with those in NC and CHB patients. Fourteen of 80 patients with CHB received 100 mg per day dose of lamivudine, and were closely followed up. Peripheral blood samples were collected from each patient before and during the administration of antiviral therapy. All LC patients had at least a 20-year history of chronic HBV infection. They received no steroid therapy before and during our observation and had the same peripheral WBC counts

Quantification of pDC

Heparin- (500 U/mL) (Sigma, St. Louis, MO) treated whole peripheral blood was incubated with a lineage cocktail of fluorescein isothiocyanate (FITC)-conjugated mAbs to CD3, CD14, CD16, CD19, CD20, and CD56 molecules; PerCP-conjugated mAb against HLA-DR; and phycoerythrin (PE)-conjugated mAb against CD123 (all reagents from BD Biosciences PharMingen, San Diego, CA) for 20 min at 4°C. Afterwards 2.0 mL of FACS^{TM} lysis solution was added for 15 min at room temperature in the dark. After centrifugation and washing twice with PBS, cells were fixed in 1% paraformaldehyde, and three-color flow cytometric analysis (FACS) was performed using FACSCalibur (Becton Dickinson, San Jose, CA). Mouse IgG1-PE was used as a negative control. Gating strategy used for analysis of pDC in peripheral blood by flow cytometry was set up as described previously (23). pDC were defined as $lineage^-$, $CD11c^-$ $HLA\text{-}DR^+$, and $CD123^+$ markers. The percentage and absolute number of pDC were calculated and compared among different groups. The absolute number of pDC was calculated using the percentage of cells with respect to the lymphocyte and monocyte absolute counts, as determined by an automated differential blood count. The timing of sample collection for analysis of circulating pDC in HBV patients with lamivudine therapy was on Day

Table I. Clinical Profiles of Subjects in Groups of HBV Infections

Parameters	NC	AH	CHB	CHB	LC
Number	25	9	80	14	27
Age (years)	32.0 ± 10.7	34.1 ± 8.6	35.0 ± 9.6	34.5 ± 7.8	48.6 ± 9.2*
Sex (male/female)	21/4	7/2	69/11	10/4	21/6
WBC	5123 ± 932	5455 ± 1344	5525 ± 1252	5480 ± 986	3635 ± 1485*
ALT U/L	28.7 ± 5.7	507.3 ± 404.5*	152.1 ± 124.9*	168.1 ± 104.6	73.2 ± 42.9
Abnormal/normal	25	9	80/0	14/0	15/12
Serum HBV DNA					
$>10^5$ copies/mL	ND	5	76	14	6
Titer range	ND	$3.1 \times 10^4 \sim 4.35 \times 10^6$	$2.6 \times 10^4 \sim 6.56 \times 10^8$	$8.9 \times 10^5 \sim 6.56 \times 10^8$	$4.5 \times 10^4 \sim 3.42 \times 10^7$
(+/−)	ND	9/0	80/0	14/0	24/3
Serologies					
HBsAg (+/−)	0/25	9/0	80/0	14/0	27/0
HBeAg (+/−)	0/25	9/0	80/0	14/0	19/8
HBcAg (+/−)	0/25	9/0	80/0	14/0	27/0

Notes. NC, healthy control; AH, acute hepatitis; CHB, chronic hepatitis B; LC, liver cirrhosis; ALT, alanine aminotransferase; WBC, white blood cell. Data are shown as mean ± standard deviation. The symbols such as + and − in brackets represent the positive and negative results determined in serum HBV antigen, or are referred to the detectable and undetectable level, respectively.
*Statistically compared with NC group, $p < 0.01$.

0 before lamivudine therapy and Day 180 after antiviral therapy.

IFN-α Induction and Detection

To evaluate the capacity of total PBMC to produce IFN-α, 5×10^5 freshly separated PBMC were incubated with an equal number of plaque forming units of UV-irradiated herpes simplex virus 1 (HSV-1) for 24 h in 96-well round-bottom plates in triplicate (17, 29). The culture supernatants were harvested and stored at −70°C until tested. Secretory IFN-α was measured by sandwich enzyme-linked immunosorbent assay in triplicate (Biosource International Inc., Camarillo, CA).

HBV DNA Assay

Serum viral load of HBV DNA was quantified for each subject using a high sensitivity fluorescent real time nested PCR kit (Da An Biological Science Co. Ltd., Guangzhou, China) and amplified in a Gene-AMP® 7900HT sequence detection system (Applied Biosystems Inc., Foster City, CA). Results were expressed as HBV DNA copies per milliliter of serum. The detection sensitivity of the PCR assay was 1×10^3 copies/mL.

Differential Blood Cell Count and Lymphocyte Subpopulations

The complete differential blood cell counts included total leukocytes, granulocytes, lymphocytes, monocytes, erythrocytes, and platelets for each subject. The numbers of $CD4^+$ and $CD8^+$ T cells, $CD19^+$ B cells, $CD16^+$ and $CD56^+$ NK cells were analyzed with a Simultest™ IMK-Lymphocyte kit (BD Biosciences, San Jose, CA), by $CD3^+/CD4^+$, $CD3^+/CD8^+$, $CD3^-/CD19^+$, $CD3^-/CD16^+$, and $CD56^+$ double staining of lysed whole peripheral blood, followed by flow cytometry with gating on the lymphocyte population according to the manufacturer's instructions. The absolute cell numbers were calculated by using the percentage of cells in relation to the lymphocyte count, which was determined by the automated differential blood count.

Statistical Analysis

For statistical analysis, we used the nonparametric (Mann–Whitney) *U* test analysis between parameters. The $p < 0.05$ was set as statistical significance.

RESULTS

Frequencies and Number of Circulating pDC in HBV-Infected Patients

Currently, there is no single molecule known to be uniquely expressed by pDC, therefore a combination of several markers (lineage$^-$, CD11c$^-$ HLA-DR$^+$, and $CD123^+$) was used to define pDC in blood (15, 16, 18, 31). The 80 CHB patients enrolled in the study had both elevation of serum ALT level and high viremia (HBV DNA viral load ranging from 2.63×10^4 to 6.56×10^8 copies/mL), suggesting chronic inflammation and active viral replication in these patients. In 25 healthy subjects, pDC represented $0.32 \pm 0.13\%$ of total PBMCs. However, pDC represented $0.19 \pm 0.09\%$ and $0.20 \pm 0.08\%$ in CHB and LC groups, respectively (Fig. 1A). The absolute number of circulating pDC was correspondingly lower in the

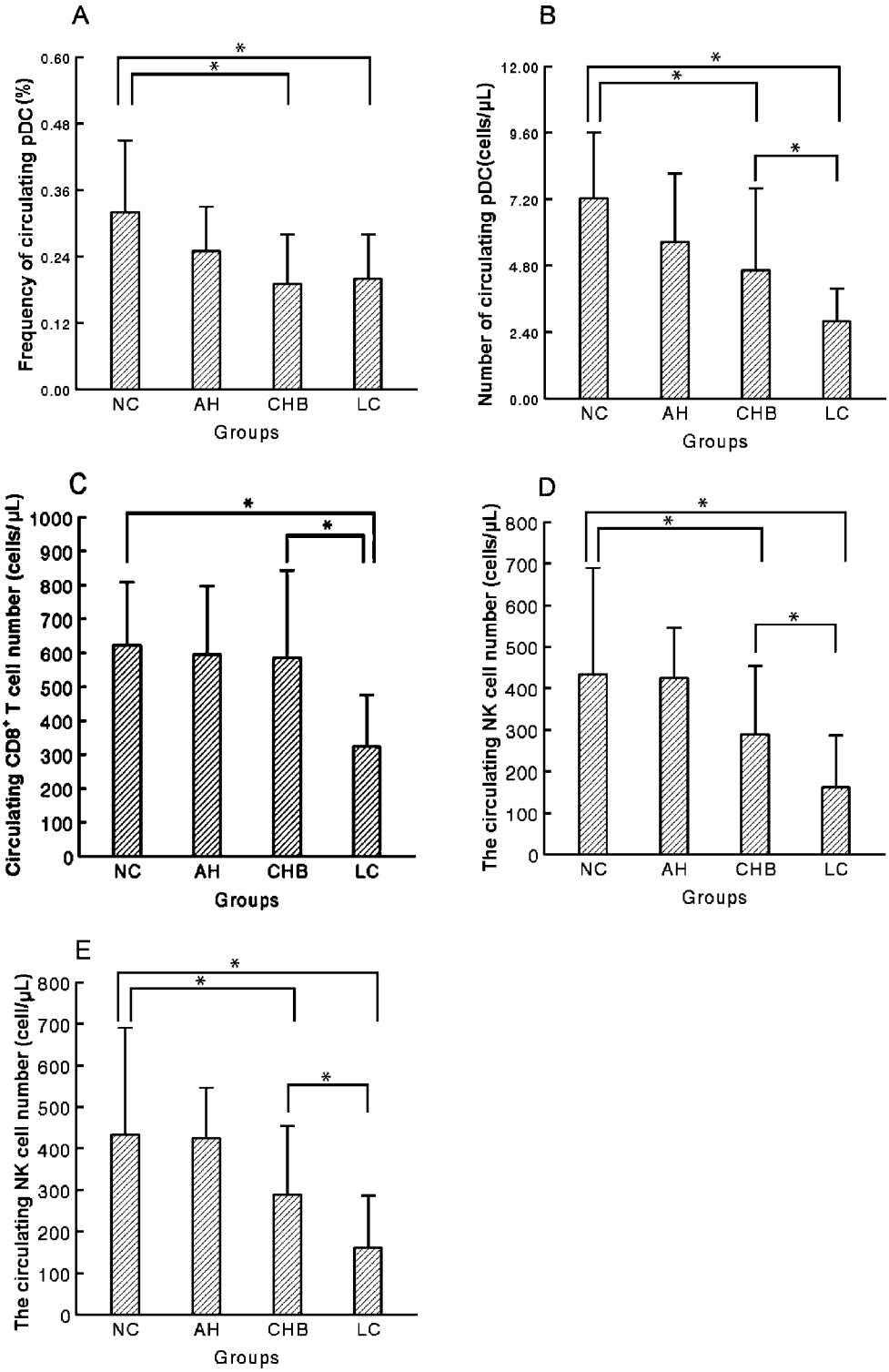

Fig. 1. Frequencies of circulating pDC, T lymphocytes, and NK cells in healthy controls (NC) and groups of patients with acute hepatitis (AH), chronic hepatitis B (CHB), and liver cirrhosis (LC). (A) and (B) represent the frequencies and absolute numbers of pDC in total PBMCs. (C) and (D) for circulating total $CD4^+$ T cells and $CD8^+$ T cells. (E) for circulating total NK cells. Data are shown as mean ± standard deviation. $^*P < 0.05$.

HBV-infected patients than in healthy controls, declining from $7.21 \pm 2.38/\mu$L cells in the healthy controls to 4.64 ± 2.94 cells/μL in CHB group and 2.78 ± 1.19 cells/μL in the LC group (Fig. 1B). Nevertheless, we found no significant difference of circulating pDC between the healthy controls and the AH patients, although the AH individuals displayed a lower level of pDC to some extent. Comparison of circulating NK cell absolute numbers in healthy donors ($n = 25$, 432 ± 258 cells/μL), patients with AH ($n = 9$, 413 ± 118 cells/μL), and CHB ($n = 80$, 292 ± 167 cells/μL) indicates that the number of NK cells is significantly reduced in chronically infected patients ($p < 0.05$) compared with healthy donors. Slightly different to NK cells in CHB patients, the absolute numbers of all peripheral $CD4^+$ T cells and $CD8^+$ T cells were found to be at the similar levels in patients with AH and CHB compared with healthy donors. However, all three of $CD4^+$ T cells, $CD8^+$ T cells, and NK cells were significantly decreased in LC group ($p < 0.01$ or $p < 0.05$) in comparison to healthy donors, but held a higher level in AH group (Fig. 1C—E).

Our study showed a significant correlation of alteration between the absolute number of pDC and the numbers of circulating $CD8^+$ T cells ($p < 0.01$) and NK cells ($p < 0.01$), but not with $CD4^+$ T cells (data not shown) in CHB patients.

IFN-α Release by PBMCs in HBV-Infected Patients

Although most blood cell types including mDC, NK cells, and other cells in PBMCs have the capacity to produce type 1 IFN in response to viral infection, pDC represent professional type 1 IFN-producing cells, producing up to 98% of type 1 IFN of total PBMC in response to stimulation with certain viruses (17). Thus, it would be desirable to isolate pDC from PBMCs of CHB patients and to directly check IFN-α production after stimulation with HBV. However, HBV is not available in a virion form and it is technically difficult to isolate pDC from so many patients. We therefore evaluated IFN-α production by same number of PBMCs *in vitro* to indirectly understand the capacity for IFN-α release by pDC. Without HSV-1 stimulation, PBMCs from all groups were found to produce very low levels of IFN-α and exhibited no differences between healthy subjects and HBV patients (Fig. 2A). IFN-α generation detected in supernatants of HSV-1-stimulated PBMCs was 789.4 ± 260.1 pg/mL in healthy controls, 540.5 ± 138.7 pg/mL in AH group, 181.1 ± 119.8 pg/mL in CHB group, and 110.1 ± 39.5 pg/mL in LC group, suggesting that there was a progressive loss of IFN-α generation in chronic HBV infection from AH, to CHB and LC status. To evaluate pDC production of IFN-α more accurately, we calculated IFN-α production at single cell level in healthy controls versus patients. We found that each pDC from healthy controls produced $114.63 \pm 28.9 \times 10^{-3}$ pg of IFN-α, significantly higher than HBV-infected patients, which ranged from $100.1 \pm 15.4 \times 10^{-3}$ pg in AH group, $42.6 \pm 16.5 \times 10^{-3}$ pg in CHB group, and $49.7 \pm 26.5 \times 10^{-3}$ pg in LC group (Fig. 3B). Our observation also showed a significant decrease of total IFN-α production of circulating PBMCs *in vitro* in both CHB and LC

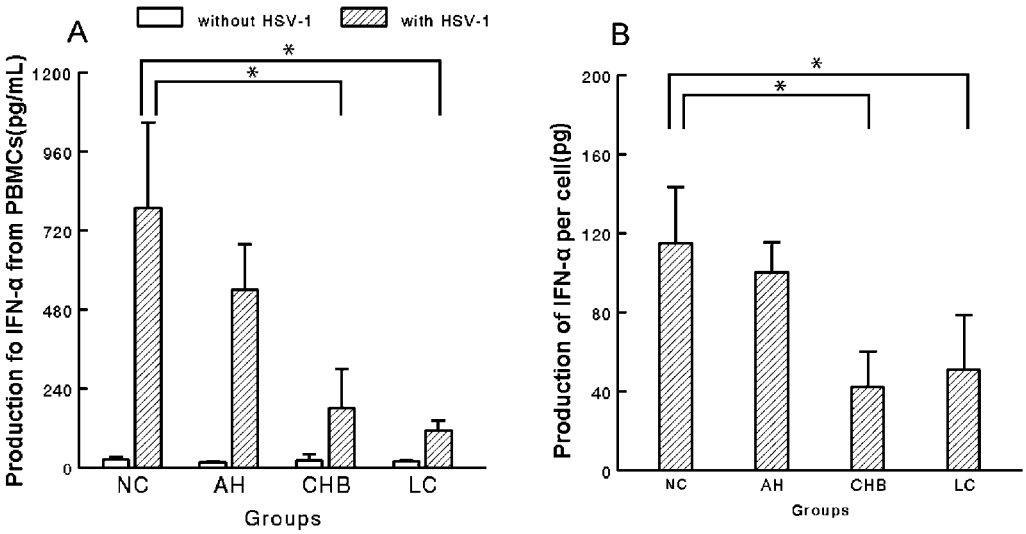

Fig. 2. IFN-α secretion by total PBMCs from HBV patients and healthy controls. Secretion of IFN-α (pg/mL) by PBMCs with and without UV-inactivated HSV-1 stimulation *in vitro*. NC, normal controls; AH, acute hepatitis; LC, liver cirrhosis. Data are shown as mean ± standard deviation. * $P < 0.05$.

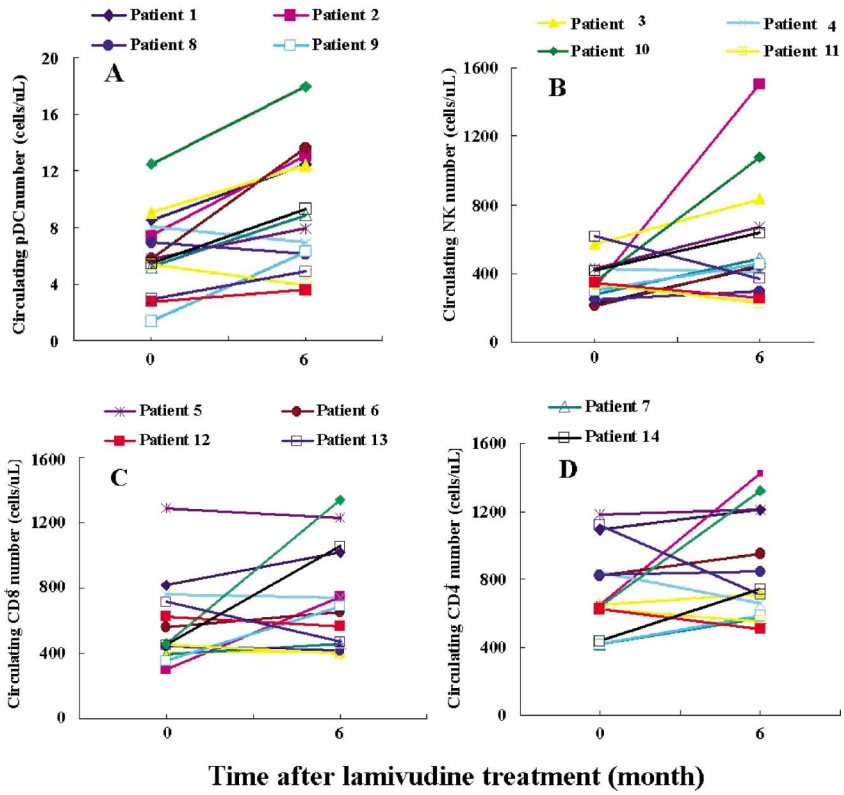

Fig. 3. Alterations of circulating pDC and T lymphocyte subsets in 14 CHB patients receiving lamivudine treatment on Days 0 and 180, respectively. (A) Circulating numbers of pDC (Day 0 vs. Day 180; $p < 0.05$ for CHB patients 1, 3, 7, and 13, $p < 0.01$ for patients 6, 9, 10, and 14). (B), (C), and (D) represent the alterations of NK cells, $CD4^+$, and $CD8^+$ T cell counts, respectively.

patients, but not in AH patients, compared to that found in healthy subjects.

Alteration of Circulating pDC in Patients Receiving Antiviral Therapy

We further analyzed 14 of 80 CHB patients, who were treated with 100 mg per day dose of lamivudine. The high level of serum HBV viremia, measured with a sensitive assay, decreased rapidly beginning within 1 month of treatment and remained at an undetectable level during the remaining period of antiviral therapy in all the 14 naïve antiviral treatment patients. In accord with the virologic response, the elevated serum ALT levels also returned to normal (<40 U/L). The alteration of pDC observed in these 14 patients could be divided into two groups. There was a significant increase of circulating pDC in 8 of 14 patients (Patients 1, 3, 6, 7, 9, 10, 13, 14) based on the comparison of data between Day 0 and Day 180 after antiviral lamuvidine therapy ($p < 0.01$ or $p < 0.05$ for all eight patients) (Fig. 3A). In parallel, IFN-α production by PBMCs returned to close to normal values in the eight patients (data not shown). Three of the eight patients (Patients 10, 13, 14) displayed a serum HBeAg seroconversion around Day 180 and had the most significant increase of pDC as well as NK cells, total circulating $CD4^+$ and $CD8^+$ T cells compared with those found on Day 0 (Fig. 3A–D). The other five patients (Patients 1, 3, 6, 7, 9) also had significant rises in pDC and were serum negative for HBeAg, but did not produce the serum antibody against HBeAg (HBeAb). However, the remaining six patients in the second group (Patients 2, 4, 5, 8, 11, 12) showed no marked increase of pDC, and three (Patients 4, 8, 11) of six even displayed a slight reduction of pDC. Correspondingly, the NK cells, $CD8^+$, and $CD4^+$ T cells also showed no significant change in this group. The six patients had an undetectable serum HBV DNA, but were serum positive for HBeAg, except for one (Fig. 3A–D).

Association of Blood pDC with Development of Nosocomial Infections in LC

Nosocomial infections (NI) frequently occur in LC patients associated with chronic HBV infection, but it is

rarely seen in CHB patients without liver cirrhosis. Since there was a further decrease of peripheral pDC in LC patients as mentioned above (14, 32), we initially analyzed the association of pDC with the occurrence of NI in our study. We defined NI as bacterial or fungal infections occurring after 48 h of hospitalization. Among the 27 LC patients in this study, 12 developed NI during the period of this investigation. Three patients displayed septicemia, five peritonitis, and four pneumonia. Ten individuals were infected with bacteria and two with fungi. The remaining 15 patients had no record of NI before and during the period of hospitalization in the study. When we compared pDC with lymphocyte numbers between these subgroups, we found that both relative frequency ($0.15 \pm 0.04\%$ vs. $0.22 \pm 0.06\%$, $p = 0.001$) and absolute number (2.25 ± 0.96 cells/μL versus 3.29 ± 1.15 cells/μL, $p = 0.019$) of pDC were significantly lower in LC patients with NI than without NI (Fig. 4A). The numbers of $CD8^+$ T cells (Fig. 4B) and NK cells (Fig. 4C) were also significantly lower in the group with NI. However, we found no difference between these subgroups in the numbers of $CD4^+$ T cells (data not shown). Therefore, reduction of blood pDC might contribute to the development of nosocomial infections in LC patients.

DISCUSSION

One of the first defense mechanisms that an infected host is able to mount against viral infections is the production of a key cytokine, type I IFNs (IFN-α/β), which is likely to limit the amplification and spread of the virus and attenuate the infection through intracellular pathways that target many steps in the viral life cycle (8, 14, 33). In addition, natural IFN-α/β-producing cells play an important role in linking innate and adaptive immunity (14). Circulating pDCs are considered to be the chief producer of IFN-α in PBMCs, therefore, the abundance of pDC in HBV-infected patients may, at least in part, reflect the

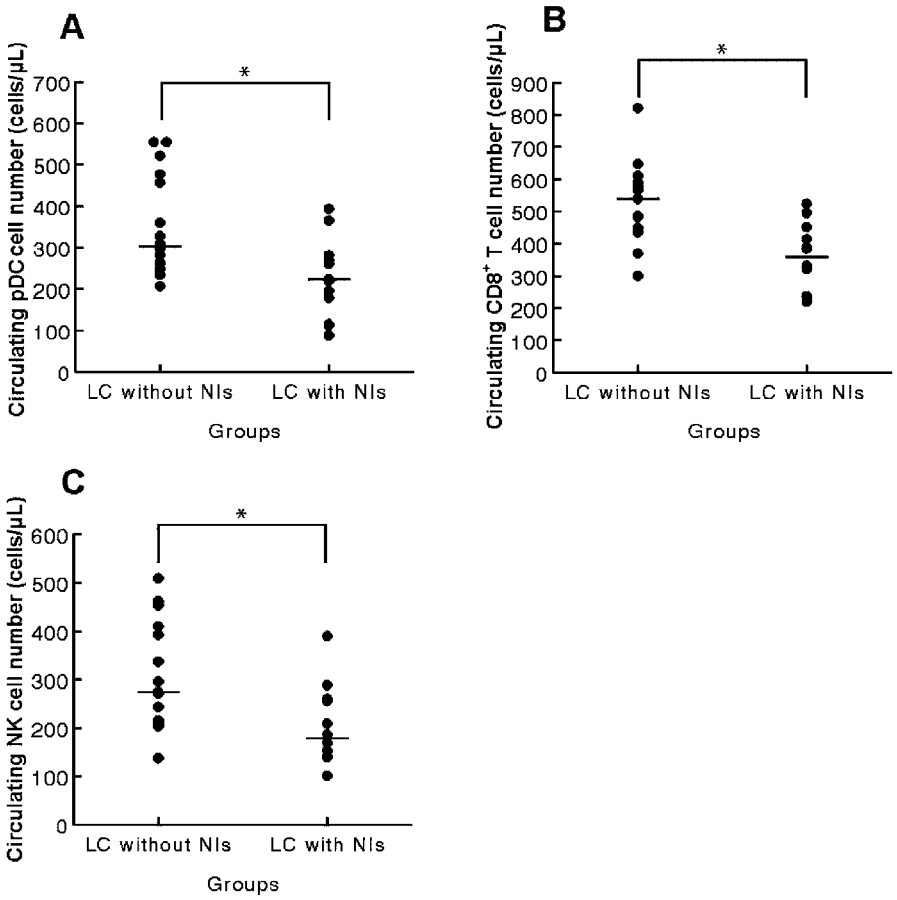

Fig. 4. Numbers of pDC and lymphocyte subsets in LC patients with and without nosocomial infections. Number of circulating pDC (A), $CD8^+$ T cells (B), and NK cells (C) in the LC groups without or with NIs. LC, liver cirrhosis; NIs, nosocomoal infections. $^*P < 0.05$ between two groups.

direct innate immune response to viral infection. In our study, we observed a progressive quantitative and qualitative impairment of pDC function in CHB and LC patients, probably from the onset of the disease in AH patients. A similar parallel correlation between the pDC count and the *in vitro* IFN-α production by PBMCs has been demonstrated in HIV-1-infected individuals (30). Thus, our observation showed that the defect of pDC seemed to be associated with the persistent HBV infection in humans. This data on pDC function based on the IFN-α production from total PBMCs *in vitro* should be interpreted with caution since we lacked direct evidence of release of IFN-α by pDC in our study. The clinical parameters including high level of viremia, serum positive HBeAg marker, and elevated ALT level indicate that all the enrolled patients in present study were at the status of active *in vivo* HBV replication and chronic hepatic inflammatory condition. Recent studies reported that DC represent targets for HBV virions (23, 24), the progressive defect of peripheral pDC in patients with established chronic HBV infection may be due in part to the prolonged exposure of these cells to viral antigens and immunosuppression by viral gene products (34, 23). Also, there may be a disorder of adaptive immune responses arising from the persistence of HBV in the circulation in these patients (6, 35, 36). In addition, the recombinant human IFN-α has been widely used for treating hepatitis B and C, but only 30% of CHB patients showed a long-term response to IFN therapy (37). Whether its efficacy is in part associated with the level of endogenous IFN-α release by pDC deserves further investigation.

It is noteworthy that the frequency of the pDC and IFN-α release by PBMCs in periphery were found to be slightly lower in AH patients than healthy subjects ($p = 0.16$ for pDC frequency, $p = 0.24$ for IFN-α production), but to be much higher than those found in CHB patients from the present limited data, suggesting that the pDC function might be minimally or not significantly affected by the virus during the acute phase of self-limited HBV infection. The finding may be in line with the evidence that vigorous polyclonal class I-restricted cytotoxic T lymphocyte (CTL) and class II-restricted CD4+ T-helper responses to HBV antigen proteins could be detected during the acute phase of HBV (1, 6).

Recent publications suggest that a prolonged duration and increased magnitude of viremia contribute to persistent T-cell hyporesponsiveness, thus control of viral replication has been considered to be essential for recovery of T-cell reactivity (9, 34). Among the 14 CHB patients who received the lamivudine therapy, 8 of them displayed a significant increase in the number and function of pDC as well as normalization of serum ALT level, suggesting lamivudine could also restore the frequency and/or function of pDC in CHB patients. This supports the notion that lamivudine does not only represent a potent inhibitor of viral replication but also has a positive immunomodulatory effect on HBV-specific CTL (9, 34, 38) and monocyte-derived DC (24). In addition, a simultaneous rise in peripheral total $CD4^+$ and $CD8^+$ T cells and NK cells, which are considered to be the principal source of IFN-γ production to further inhibit HBV replication (39, 40), was observed in the eight CHB patients. Taken together, our findings suggest that the synergistic recovery of both innate and adaptive immunity may be, to some extent, associated with the control of HBV replication (5). By contrast, we found pDC numbers to be unaltered in three of the remaining six patients and even decreased in the other three patients. The underlying mechanisms have not been disclosed yet, but may reflect differences in immunocompetence (6, 9), differences of HBV genotype (41–43), and other reasons (18, 44).

Compared with CHB patients, LC individuals had a further defect of pDC, together with the simultaneous decrease of peripheral total $CD4^+$ T cells, $CD8^+$ T cells, and NK cells. We observed that the alteration of the components correlated with the occurrence of nosocomial infections in LC patients. Besides the aforementioned reasons responsible for the reduction of pDC as described in CHB patients, the age-related changes that probably have an impact on the maturation, the host immune response may be another principal reason to contribute to the decline of circulating pDC and $CD4^+$ T cells in older LC patients than CHB patients in our study (45, 46). Soumelis *et al*. have recently reported that the depletion of pDC was linked with the development of opportunistic infection in AIDS patients (29). In addition, Teig *et al*. recently demonstrated that there is a direct correlation between the number of pDC and IFN-α production (46). Accordingly, our initial data is likely to show an apparent predisposition to NI in LC patients with significant impairment of pDC. However, it remains to be investigated whether this alteration of pDC, together with the collapse of adaptive immunity (6), synergistically enhance the susceptibility to NI in LC individuals (45).

In conclusion, this study showed a defect of circulating pDC in chronic HBV individuals and a correlation of the frequency of pDC with HBV persistence during disease progression, suggesting that pDC may play an important role in pathogenesis of chronic hepatitis. It is believed that a complete understanding of pDC function and its interplay with NK cells, $CD8^+$ T cells, and $CD4^+$ T cells in innate and adaptive immune responses will help to establish a platform for development of more efficient therapeutic strategy against chronic HBV infection.

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