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Potential role of HBV DNA-induced CD8^{high} T cell apoptosis in patients with systemic lupus erythematosus and rheumatoid arthritis

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Abstract

Objective: To investigate the potential role of hepatitis B virus (HBV) DNA-induced CD8^{high} T cell apoptosis in patients with systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA).

Methods: The activity and HBV seropositivity rates of patients with SLE and RA were determined. The proportions of T cell subgroups were detected by fluorescence-activated cell sorting. The apoptosis of T cell subgroups was detected after peripheral blood mononuclear cells were stimulated with HBV DNA.

Results: The HBV infection rate was higher in patients with RA than in patients with SLE. Current or previous HBV infection was more common among patients with inactive SLE than among those with active SLE. Conversely, previous or current HBV infection was more common among patients with active RA than among those with inactive RA. CD4^{-}CD8^{high} T cell counts were higher among patients with active SLE than in those with inactive SLE. However, CD4^{-}CD8^{high} T cell counts were lower in patients with active RA patients than in those with inactive RA. HBV DNA increased the apoptosis of CD4^{-}CD8^{high} T cells.

Conclusion: HBV DNA-induced CD8^{high} T cell apoptosis appears to play different roles in SLE and RA.

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Introduction

Increasing evidence supports of the involvement of hepatitis B virus (HBV) infection in the pathogenesis and progression of autoimmune diseases. Despite the existence of a preventive vaccine, HBV infection remains prevalent, having caused approximately 250 million infections globally.[1] Systemic lupus erythematosus (SLE) is an autoimmune disease that can affect the skin mucous membrane, skeletal muscles, heart, kidneys, respiratory system, nervous system, and blood system. SLE is characterized by the excessive production of a variety of antinuclear antibodies involved in the immune response. Rheumatoid arthritis (RA) is a serious inflammatory synovitis of multiple systems, and it often affects the small joints of the hands and feet. Symmetry is a primary characteristic of RA. SLE and RA share several clinical manifestations, serological profiles, and immunological characteristics. These two conditions remain widely recognized rheumatic diseases of unknown etiology in which extensive immune dysfunction has been reported.[2] Infective agents have been identified as key factors of autoimmune diseases.[3] Different T cell subgroup responses may be either beneficial or detrimental to patients infected with HBV. $CD8^{+}$ T cells are the principal contributing factors of HBV elimination, but they play different roles in the active stages of autoimmune diseases.[4,5] Some researchers reported that HBV infection or HBV vaccination is associated with the induction of autoimmune diseases.[6,7] On the contrary, other researchers described the putative protective role of HBV infection against some autoimmune disorders.[8] Therefore, the relationship between HBV infection and autoimmune diseases remains unclear. HBV might play different roles than a triggering agent in autoimmune diseases.

In this study, we investigated the prevalence of HBV infection in patients with SLE and RA and evaluated the prevalence of current ($HBsAg^{+}$) and previous HV infection ($HBsAg^{-}HBcAb^{+}$) in the different stages of SLE and RA patients. The proportion of $CD4^{-}CD8^{high}$ T cells among lymphocytes differed between the inactive and active stages of SLE and RA patients, and HBV DNA-induced apoptosis of $CD4^{-}CD8^{high}$ T cells was analyzed. These results revealed that HBV DNA-induced $CD8^{high}$ T cell apoptosis could be beneficial in SLE but deleterious in RA.

Materials and methods

Patients and samples

Clinical case data covering the period of 2012 to 2019 were obtained from the Department of Rheumatology and Immunology of the First Affiliated Hospital of Anhui Medical University (Hefei, China). All participants provided verbal informed consent before their data were included in the study. Regarding the diagnostic criteria of SLE, SLEDAI 2000 scores of <6 and ≥ 6 indicated inactive

and active disease, respectively.[9] According to the DAS28-ESR standard, scores of ≤ 3.2 and >3.2 indicated inactive and active disease, respectively.[10] Enzyme-linked immunosorbent assay for serological markers (HBsAg, HBsAb, HBeAg, HBeAb, and HBcAb) was used to evaluate HBV infection. In addition, HBV test data and peripheral blood samples were collected for healthy controls. This study was approved by the ethics committee of Anhui Medical University (No. 20140057).

Flow cytometry

Peripheral blood was mixed with PBS at a 1:1 ratio, Ficoll (GE Healthcare, Chicago, IL, USA) was added, and the mixture was centrifuged at $400 \times g$ for 40 minutes. The isolated PBMCs were washed twice with PBS, and APC-Cy7-CD3, FITC-CD4, and BV421-CD8 antibodies (all from BD Biosciences, Franklin Lakes, NJ, USA) were added. Five microliters of each antibody were added, and the samples were incubated in the refrigerator for 30 minutes. Unbound antibodies were washed with PBS, and then 1% paraformaldehyde was added before analysis by flow cytometry (CytoFLEX, Beckman-Coulter, Brea, CA, USA).

Cell culture and HBV DNA extract

HepG2.2.15 cells (iCell Bioscience Inc, Shanghai, China) were cultured with DMEM (HyClone, Logan, UT, USA) with 10% FBS and 380 μg/mL G418 (Sigma-Aldrich). First, the supernatant of HepG2.2.15 cells was concentrated using a PEG Virus Precipitation Kit (BioVision, Abcam, Cambridge, UK), placed in a refrigerator overnight, and centrifuged, and the precipitate was resuspended in PBS. Next, HBV DNA was captured using a DNA Blood Mini Kit (QIAamp, Qiagen, Hilden, Germany) in accordance with the manufacturer's protocol. Peripheral blood mononuclear cells (PBMCs) were separated from blood samples with Ficoll cultured with RPMI-1640 (1 mL; Gibco, Thermo Fisher Scientific, Inc., Waltham, MA, USA) containing 10% FBS, incubated at 37°C for 2 hours, and then stimulated with HBV DNA (250 ng/mL) for 48 hours.

Apoptosis of immune cells

An Annexin-V/7-AAD apoptosis kit (Best Bio, Shanghai, China) was used to analyze cell apoptosis based on the manufacturer's protocol. Cells (5×10^5) incubated with HBV DNA were harvested and washed with cold 1× PBS. APC-Cy7-CD3, FITC-CD4, and BV421-CD8 antibodies (5 μL each) were added. The cells were resuspended in 500 μL of 1 × binding buffer, and 5 μL of Annexin V-PE and 5 μL of 7-AAD (both from Best Bio) were then added to the cell mixture, followed by incubation in the dark at 4°C for 10 minutes. Finally, apoptosis was detected by flow cytometry (Beckman-Coulter).

Statistical analysis

SPSS 17.0 software (IBM Corp., Armonk, NY, USA) was used, and comparisons of quantitative data between two groups were performed using the *t*-test. Qualitative data were analyzed by analysis of variance. Significance was indicated by $P < 0.05$.

Results

The HBV infection rate was higher in patients with RA than in patients with SLE

In total, the clinical data of 1040 patients with SLE, including 956 women and 84 men with an average age of 38.4 years and an average age at onset of 32.2 years, were collected. Data were also collected for 1000 patients with RA, including 805 women and

195 men with an average age of 54.8 years and an average age at onset of 44.7 years. Data were additionally collected for 29 healthy controls. The HBsAg seropositivity rate was 1.3% in patients with SLE, which was significantly lower than that in patients with RA (4.3%, $P < 0.01$). The rate of HBeAg seropositivity was 0.3% in patients with SLE, versus 1.2% in patients with RA ($P < 0.05$). The HBeAb seropositivity rate was 5.6% in patients with SLE, which was a significantly lower than that in patients with RA (8.1%, $P < 0.05$). In patients with RA, the HBcAb seropositivity rate was 22.8%, which was significantly higher than the rate in patients with SLE (14.3%, $P < 0.01$; Figure 1).

Current and previous HBV infection rates according to the stage of SLE and RA

We next analyzed the rates of current ($HBsAg^+$) and previous HBV infection ($HBsAg^-HBcAb^+$) in patients with SLE or RA. According to the HBV test, 14 of 1040 patients with SLE and 42 of 1000 patients with RA were $HBsAg^+$. Moreover, 141 patients with SLE and 168 patients with RA were $HBsAg^-HBcAb^+$. In SLE, 71.4% of patients with $HBsAg^+$ were considered to have inactive disease, whereas 28.6% had active disease. In RA, 4.8% of patients with $HBsAg^+$ had inactive disease, whereas 95.2% of patients had active disease. We also analyzed the disease activity of $HBsAg^-HBcAb^+$ patients with SLE and RA. We found that 61.7% of patients with previous HBV infection had inactive SLE, whereas 4.2% of patients with previous HBV infection had inactive RA. Therefore, both existing and previous HBV infection were more common in patients with inactive SLE or active RA (Figure 2).

The proportion of $CD4^-CD8^{high}$ T cells among lymphocytes according to the disease activity of SLE and RA

To investigate the differences in T cell subgroups between the two diseases, patients with SLE were divided into inactive and active groups The proportions of T cell subgroups, including $CD3^+$, $CD4^+$ $CD8^-$, $CD4^-$ $CD8^+$, $CD4^-CD8^{high}$, $CD4^-CD8^{low}$, $CD4^-CD8^-$, and $CD4^+CD8^+$ T cells, in

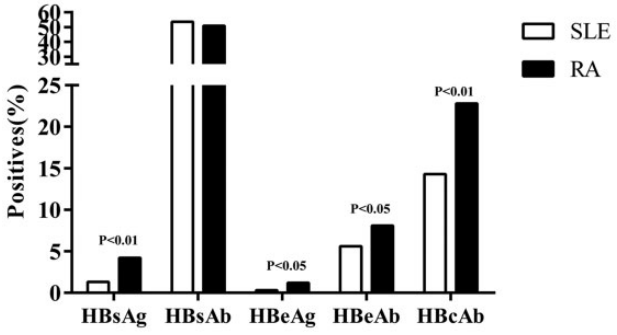

Figure 1. Comparison of the prevalence of HBV infection in patients with SLE and RA. The HBsAg seropositivity rate was lower in patients with SLE (1.3%) than in those with RA (4.3%, $P < 0.01$). The HBeAg seropositivity rate was significantly lower in patients with SLE (0.3%) than in those with RA (1.2%, $P < 0.05$). The HBeAb seropositivity rate was significantly lower in patients with SLE (5.6%) than in those with RA (8.1%, $P < 0.05$). In total, 22.8% of patients with RA had detectable HBcAb levels, which was significantly higher than the rate in patients with SLE (14.3%, $P < 0.01$).
HBV, hepatitis B virus; SLE, systemic lupus erythematosus; RA, rheumatoid arthritis.

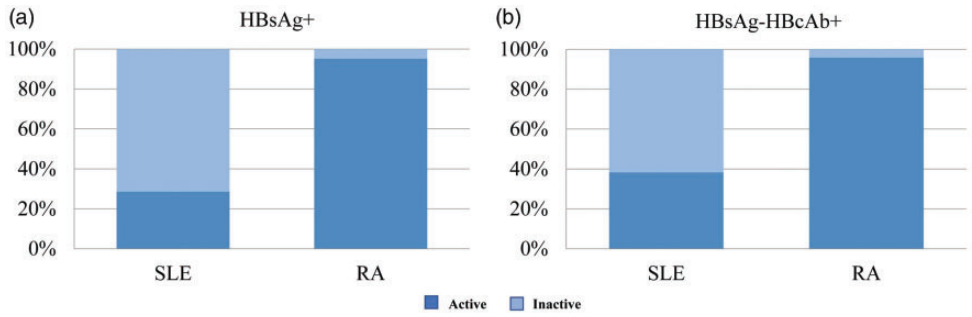

Figure 2. Rates of current or previous HBV infection in patients with SLE and RA. (a) In total, 71.4% of patients with SLE with HBsAg seropositivity had inactive disease, whereas 28.6% had active disease. In RA, 4.8% of patients with HBsAg seropositivity had inactive disease, whereas 95.2% had active disease and (b) In SLE, 61.7% of $HBsAg^{-}HBcAb^{+}$ patients had inactive disease, whereas in RA, 4.2% of $HBsAg^{-}HBcAb^{+}$ patients had inactive disease.
HBV, hepatitis B virus; SLE, systemic lupus erythematosus; RA, rheumatoid arthritis.

different disease activity stages were further analyzed. The results illustrated that the proportions of $CD4^{-}CD8^{+}$ and $CD4^{-}CD8^{high}$ T cells was significantly higher in patients with active SLE than in those with inactive SLE ($P < 0.05$; Figure 3). However, the proportions of $CD8^{+}$ and $CD8^{high}$ T cells were significantly lower in patients with active RA than in patients with inactive RA and healthy controls ($P < 0.05$; Figure 4).

HBV DNA induced the apoptosis of $CD4^{-}CD8^{high}$ T cells

With persistent HBV infection, the levels of various antigens and antibodies fluctuate, whereas HBV DNA persists. To observe the effect of HBV DNA on T cells in patients with SLE or RA *in vitro*, HBV DNA was extracted from the supernatant of HepG2.2.15 cells and then cultured with PBMCs from healthy individuals. The apoptosis rate of $CD4^{-}CD8^{high}$ T cells was analyzed after 48 hours of culture. The results (Figure 5) illustrated that the apoptosis of $CD4^{-}CD8^{high}$ T cells was significantly increased by HBV DNA stimulation ($P < 0.05$).

Discussion

Previous studies demonstrated that patients with SLE have a lower prevalence of HBV infection, whereas patients with RA have a higher rate of HBV infection.[11,12] However, other studies reported contradictory findings.[13] To investigate the prevalence of HBV infection in patients with SLE and RA, we first collected data from 2040 subjects residing in different cities in Anhui Province. The first result of the study was that the HBV infection rate was significantly lower in patients with SLE than in patients with RA. This observation is in line with some previous reports.[11,13] The different infection rates were notably related to age, geographical distribution, and genotype. Then, we analyzed the relationship between the HBV infection status and disease activity. We found that 71.4% of patients with $HBsAg^{+}$ SLE had inactive disease, whereas $HBsAg^{-}HBcAb^{+}$ patients comprised the majority of patients with inactive SLE. The opposite pattern was observed in RA. We suggest two theories to explain the results of the survey. One hypothesis is that specific autoimmune diseases may protect against or increase the risk of HBV infection. The second

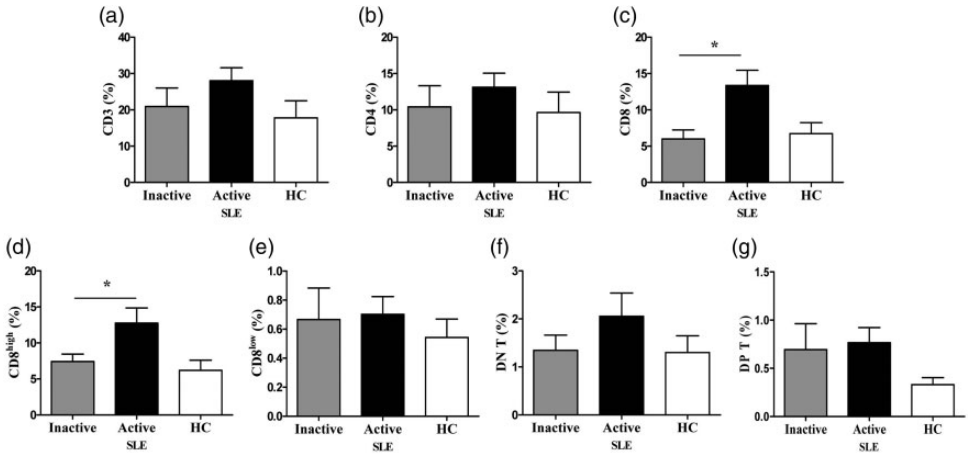

Figure 3. Proportions of T cells in patients with inactive and active SLE. The proportions of (a) $CD3^+$, (b) $CD4^+$, (c) $CD8^+$, (d) $CD4^-CD8^{high}$, (e) $CD4^-CD8^{low}$, (f) $CD4^-CD8^-$ and (g) $CD4^+CD8^+$ T cells in patients with active and inactive SLE are presented ($P < 0.05$).
SLE, systemic lupus erythematosus.

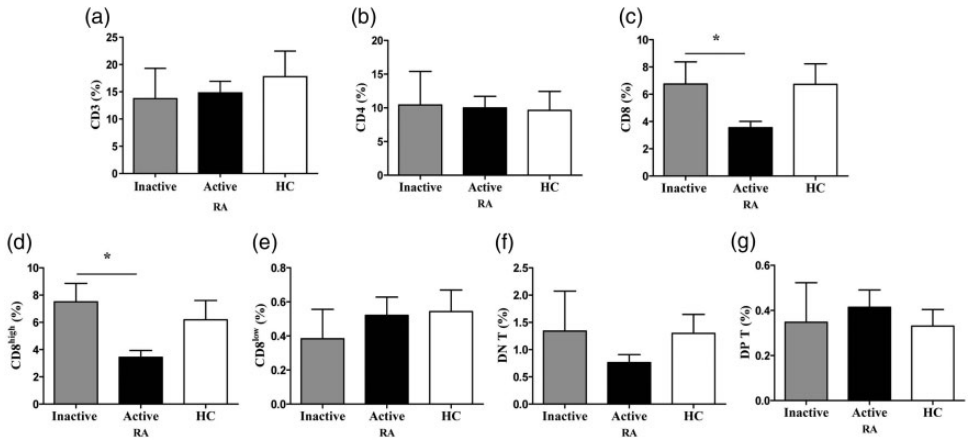

Figure 4. Proportions of T cells in patients with inactive and active RA patients. The proportions of (a) $CD3^+$, (b) $CD4^+$, (c) $CD8^+$, (d) $CD4^-CD8^{high}$, (e) $CD4^-CD8^{low}$, (f) $CD4^-CD8^-$ and (g) $CD4^+CD8^+$ T cells in patients with active and inactive RA are presented ($P < 0.05$).
RA, rheumatoid arthritis.

hypothesis is that HBV infection protects against SLE or aggravates RA. The first theory was supported by the secretion of interferon (IFN)-α and inflammatory factors. IFN-α plays a critical role in the pathogenesis and perpetuation of SLE, and this signaling protein can enhance the natural immune response to HBV. Meanwhile, RA is a chronic inflammatory disease leading to joint destruction.[14,15] Elevated levels of inflammatory cytokines, especially interleukin-6 and tumor necrosis factor-α, may have important roles in the course of hepatitis B-induced liver injury. In our

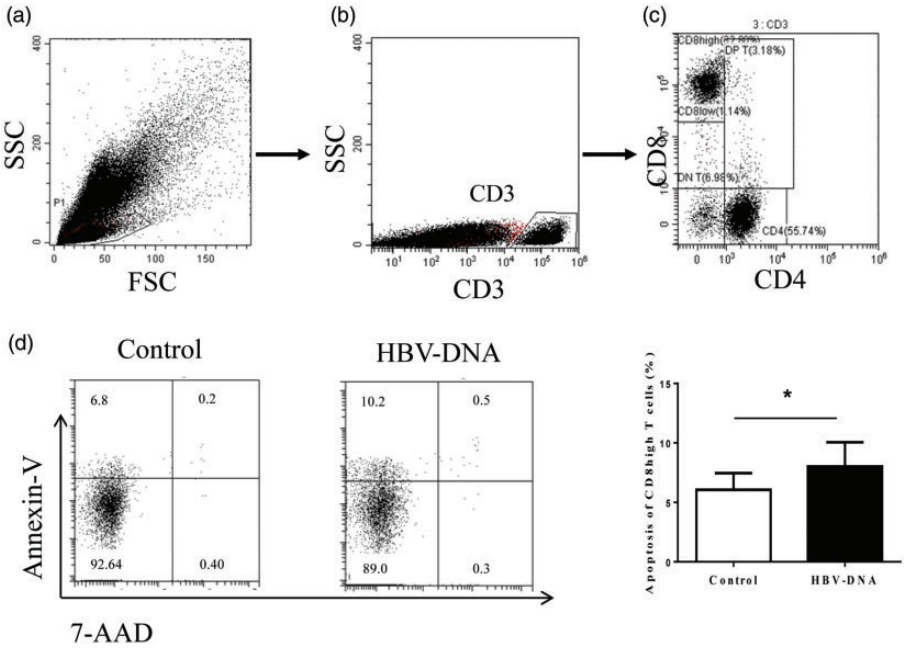

Figure 5. HBV DNA induced the apoptosis of $CD8^{high}$ T cells. The apoptosis of $CD4^{-}CD8^{high}$ T cells was gated and measured by flow cytometry after incubating PBMCs with 250 ng/mL HBV DNA for 48 hours ($P < 0.05$). (a) PBMCs were gated on FSC and SSC light plots, (b) $CD3^{+}$ and (c) $CD4^{-}CD8^{high}$ T cells were identified and (d) the apoptosis of $CD4^{-}CD8^{high}$ T cells was measured ($P < 0.05$).
HBV, hepatitis B virus; PBMC, peripheral blood mononuclear cell.

story, it was clear that patients with prior or current HBV were more likely to have inactive SLE or active RA; thus, we conjectured that in RA, disease activity was milder in patients without HBV infection. However, disease activity may be milder in patients with SLE and prior or current HBV infection. In other words, HBV might exacerbate RA symptoms and alleviate SLE symptoms. At the same time, the virus stimulates acquired immune cells to induce the activation of B cells and the production of autoantibodies, such as rheumatoid factor, thus inducing and maintaining the pathogenesis of RA. However, HBV infection does not have negative effects on all autoimmune diseases, and exploration of the relationship between infection and allergic disease stems from the hygiene hypothesis theory that suggests that, based on one view, infection can also prevent autoimmune diseases and protect the body, thus supporting the role of HBV infection in the pathogenesis of SLE. Therefore, we speculate that HBV infection has a protective effect against the development of SLE and adverse effects on RA.

Additional evidence supporting the second hypothesis was found in this study. The percentages of $CD4^{-}CD8^{+}$ T cells, especially $CD4^{-}CD8^{high}$ T cells, differed according to disease activity. A quantitative and functional increase in $CD8^{+}$ cytotoxic T lymphocytes is highly correlated with SLE disease activity.[16] However, CD8-deficient mice developed disease with an increased incidence and greater severity, suggesting that $CD8^{+}$ T cells may have a protective or regulatory role in the development of RA.[17] In our result, the

proportion of $CD4^{-}CD8^{high}$ T cells was higher in patients with active SLE than in those with inactive SLE, whereas the opposite trend was observed in RA. Therefore, we hypothesized that $CD4^{-}CD8^{high}$ T cells play different roles in these two autoimmune diseases. Specifically, a low proportion of $CD4^{-}CD8^{high}$ T cells is one factor that tends to alleviate SLE disease, whereas a reduced proportion of $CD4^{-}CD8^{high}$ T cells can promote the transformation of inactive RA to the active phase. The usage of antiviral drugs in patients with chronic HBV could induce the serological transformation of HBV, whereas HBV DNA may persist. In contrast to HepG2 cells, HepG2.2.15 cells transfected with HBV DNA can integrate the DPP7 gene and participate in apoptosis induction, suggesting that HBV DNA promotes cell apoptosis.[18] To assess this possibility, we used HBV DNA to stimulate PBMCs from healthy individuals. The results indicated that stimulation with HBV DNA significantly increased the apoptosis of $CD8^{high}$ T cells. We speculate that HBV DNA promotes $CD8^{high}$ T cell apoptosis in patients with SLE, thereby promoting disease remission. Meanwhile, HBV DNA promoted $CD8^{high}$ T cell apoptosis in patients with RA, thereby aggravating disease. Our data revealed a previously ignored effect of HBV DNA on $CD8^{high}$ T cells.

$CD8^{+}$ T cells play important roles in opposing or promoting autoimmune disease through cytokines or inappropriate apoptosis induction in target cells.[19,20] $CD8^{+}$ T cell depletion is an important biological marker condition associated with the alleviation of type I diabetes.[21] $CD8^{+}$ T cell counts are elevated in patients with antineutrophil cytoplasmic antibody-associated vasculitis, indicating an adverse prognostic trend.[22] Activated $CD8^{+}$ T cells accumulate in the joints of patients with RA, increasing the severity of disease.[23] Overall, the role of CD8 T cells in autoimmune diseases deserves further analysis. However, this study had some limitations. Whether changes in the proportion of $CD8^{high}$ T cells can reverse the progression of disease in patients remains to be elucidated.

In this study, we analyzed $CD8^{high}$ T cells in both the inactive and active phases of SLE and RA. It was concluded that the proportion of $CD8^{high}$ T cells was significantly higher in patients with active SLE than in those with inactive SLE but significantly lower patients with active RA than in those with inactive RA. Reducing $CD8^{high}$ T cell counts should be beneficial in patients with inactive SLE.

Meanwhile, current or previous HBV infection was more common in patients with inactive SLE or active RA. The percentage of $CD4^{-}CD8^{high}$ T cells was elevated in patients with active SLE; therefore, we deduced that HBV DNA induced the apoptosis of $CD4^{-}CD8^{high}$ T cells, which played a protective role in patients with SLE. However, apoptosis of $CD4^{-}CD8^{high}$ T cells had a deleterious effect in patients with RA.

Conclusion

HBV infection, through the induction of $CD8^{high}$ T cell apoptosis, might protect against the eventual development of SLE disease but induce the development of RA.

Declaration of conflicting interest

The authors declare no conflicts of interest.

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