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• *BRIEF REPORTS* •

Therapeutic effect of autologous dendritic cell vaccine on patients with chronic hepatitis B: A clinical study

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Abstract

AIM: To investigate the therapeutic effect of autologous HBsAg-loaded dendritic cells (DCs) on patients with chronic hepatitis B.

METHODS: Monocytes were isolated from fresh peripheral blood of 19 chronic HBV-infected patients by Ficoll-Hypaque density gradient centrifugation and cultured by plastic-adherence methods. DCs were induced and proliferated in the culture medium with recombinant human granulocyte-macrophage-colony- stimulating factor (rhGM-CSF) and human interleukin-4 (rhIL-4). DCs pulsed with HBsAg for twelve hours were injected into patients subcutaneously twice at intervals of two weeks. Two patients received 100 mg oral lamivudine daily for 12 mo at the same time. HBV-DNA and viral markers in sera of patients were tested every two months.

RESULTS: By the end of 2003, 11 of 19 (57.9%) patients had a clinical response to DC-treatment. HBeAg of 10 (52.6%) patients became negative, and the copies of HBV-DNA decreased $10^{1.77\pm2.39}$ averagely (t = 3.13, P<0.01). Two cases co-treated with DCs and lamivudine had a complete clinical response. There were no significant differences in the efficient rate between the cases with ALT level lower than 2×ULN and those with ALT level higher than 2×ULN before treatment (χ^2 = 0.0026).

CONCLUSION: Autologous DC-vaccine induced *in vitro* can effectively suppress HBV replication, reduce the virus load in sera, eliminate HBeAg and promote HBeAg/anti-HBe transformation. Not only the patients with high serum ALT levels but also those with normal ALT levels can respond to DC vaccine treatment, and the treatment combining DCs with lamivudine can eliminate viruses more effectively.

Key words: HBsAg; Dendritic cells

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INTRODUCTION

It is generally accepted that dendritic cells (DCs) are the most efficient and powerful antigen presenting cells (APC) and play a key role in exciting immune reaction. They also have a close relation with immunologic tolerance. At present, it has been known from studies on the mechanism of hepatitis B persistence that the cause of poor response or incompetent response of patients with chronic hepatitis B to HBV is mainly the function defection of APC, especially DCs[1-4]. In the past three years, we have done much basic research on the relation between DCs and the persistence of hepatitis B and anti-HBV immunity. The results showed that peripheral blood mononuclear cells (PBMC) could be induced by cytokine GM-CSF and IL-4 into DCs with a powerful antigen presenting function. When pulsed with HBsAg, DCs could induce HBsAg-specific cytotoxic T lymphocytes (CTL) to kill target HepG2-S cells expressing HBsAg, and these DCs have good therapeutic effects on nude mice tumors expressing HBsAg[5]. We aimed to investigate the therapeutic effect of HBsAg-pulsed DCs on patients with chronic hepatitis B on the basis of *in vitro* and animal experiments and to promote clinical application of this therapy.

MATERIALS AND METHODS

Subjects

We randomly selected 19 patients (16 males and 3 females) with chronic hepatitis B from our in-patient and out-patient departments between August 2001 and August 2002. The diagnostic criteria were coincident with "The Program of Prevention and Care for Viral Hepatitis" revised in 2000[6]. The ages of patients ranged from 17 to 56 years (average, 30.2 years), the disease course ranged from 1 to 9 years (average, 3.7 years). They were persistently positive for HBsAg, HBeAg and HBV-DNA. The copies of HBV-DNA were higher than 10^5 per milliliter before treatment. They had no sign of liver cirrhosis and infection with other hepatitis viruses or serious complications. All had not received anti-virus treatment at least for one year except for two who received lamivudine co-treatment.

Table 1 Clinical response of 19 patients treated by DC vaccine

Patients	Cases	Difference in copies of HBV-DNA between pre- and post-treatment (mean±SD)	Rate of negative for HBeAg (%)	Rate of HBeAg/anti-HBe conversion (%)
Responder	11	3.074±2.397	91 (10/11)	45.5 (5/11)
Non-responder	8	-0.021±0.495	0 (0/8)	0 (0/8)
All patients	19[1]	1.771±2.39[b]	52.6 (10/19)	26.3 (5/19)

[1]: total response rate = 57.9% (11/19); [b]P<0.01 (pair-matching t test: t = 3.13): Comparison analysis of the HBV-DNA copies of all patients between pre- and post-treatment.

DC vaccine

DCs were expanded as described by Li *et al*[7], with minor modifications. Briefly, PBMC was isolated from 60 mL of fresh citrate-treated peripheral blood obtained by venipuncture from patients using Ficoll-Hypaque density gradient centrifugation. After two washes with Hank's buffer, the cells (6×10^6/well) were seeded in 6-well plates and cultured in 1640 medium for 2 h. The nonadherent cells were gently removed, the fresh complete culture medium with 100 mL/L human AB serum supplemented with 1 000 U/mL of rhGM-CSF and 500 U/mL of rhIL-4 was added to the wells. Half of the medium was refreshed and cytokine at the half concentration was added every 2 d. On the 7th d of incubation, the suspended cells were collected (the induced DCs), counted and pulsed with HBsAg at 5 μg/mL 12 h before injection into patients, then washed twice with saline and injected subcutaneously into the patients.

Every recipient had the acknowledgment of and agreed with the whole experimental process. They were assigned the patient's agreement. All procedures were performed according to good laboratory practice standards.

Therapeutic schedule and clinical monitoring

Experimental subjects were given two standard injections of DC vaccine at two-week intervals. Post-vaccination follow-up for evaluation of vaccine efficacy lasted for at least one year. Blood samples were collected from subjects every two months, and liver function, HBsAg/HBsAb, HBeAg/HBeAb, HBc-IgG/IgM, HBV-DNA were detected.

Statistical analysis

Efficacy was defined as occurrence of sustained loss or 50% pre-vaccination-level decrease of HBV-DNA, negative for HBeAg, or sero-reverse of HBeAg/anti-HBe, normal transaminase. The data were analyzed by pair-matching t test and χ^2 test. The efficacy of DC treatment and related factors were analyzed.

RESULTS

DC morphology

On the second or third day, more and more adherent cells began to suspend and cluster. On the 4th or 5th d, the cells became larger and dendritic and on the 6th or 7th d, more and more dendritic cells were induced and became larger and larger, but at the same time few dead or apoptosis cells which reflected light weakly and had particles in cytoplasm, could be observed.

The quantity or quality of DCs was different in different patients. In our experiment, we found that DCs from the patients who had a good response to DC-treatment were generally in good condition, with the typical characteristics of dendritic cells.

Response reaction

Post-vaccination follow-up of these 19 patients lasted till the end of 2003. The results (Table 1) showed that 11 patients had a response to the treatment, among them 3 cases lost HBV-DNA, 2 cases had their HBV-DNA decreased to 5×10^2 copies/mL. The two patients co-treated with DC-vaccine and lamivudine had a relatively complete response: one lost HBV-DNA and had HBeAg/anti-HBe seroconversion, the other only had loss of HBV-DNA. None of these 11 patients had HBsAg/anti-HBs conversion.

Analysis of the relation between changes of alanine transaminase (ALT) and response reaction

The results are shown in Table 2. In the 3 cases whose serum ALT levels were normal, one had clinical response, and all had normal ALT. In the 12 cases whose serum ALT levels were two times higher than the upper limit of normal level (>2×ULN), 7 had clinical response and their ALT became gradually normal during follow-up, and others had no changes in ALT or clinical response. Among the 4 patients whose serum ALT levels were between normal and 2×ULT, 3 cases whose ALT was >2×ULN had response.

Table 2 Relation between pre-treatment ALT and result of clinical response (cases)

Patients	ALT		
	Normal	Nomal-2×ULN	>2×ULN
Responder	1	3	7
Non-responder	2	1	5

DISCUSSION

It has been shown in basic and clinical researches that the function defect of DCs is the important cause of hepatitis B chronicity and carrier status[8]. So the active immunotherapy based on DCs may become a prospective method to treat chronic hepatitis B. In this study, we treated chronic hepatitis B patients with autologous HBsAg-pulsed DCs and did the strict follow-up[9-11].

In our experiment, we found that DCs induced in hepatitis patients were in a relatively bad condition compared to healthy donors. Furthermore, DCs in 11 patients who had clinical response, were relatively better than those who had no response. These results indicate that the quality of

DCs might be very vital to the therapy and pathogenesis of chronic hepatitis B. The mechanism is still not very clear, maybe it has some relation with the chronic infection of PBMC with HBV.

At present, the main anti-HBV medicines, such as interferon-α (IFN-α) or lamivudine, have a good effect only on patients with high ALT and HBV replication, but a poor effect on HBV-carriers with normal ALT[12-14]. Our results showed that among the 12 patients with 2×ULN of pre-treatment ALT, 7 cases had clinical response and normal ALT during later follow-up, the other 5 patients had neither response nor reduction of ALT. It is suggested that ALT reversion to normal level may be the necessary condition of these patients with clinical response and high pre-treatment ALT. Among the other 7 cases with ALT lower than 2×ULN, 4 had clinical response with different changes in ALT levels. These results indicate that there were some different mechanisms of response between the two groups, and the patients with ALT lower than 2×ULN also had response to the HBsAg-DC treatment. These findings suggest that HBsAg-DC therapy can be adapted by more and more patients with chronic hepatitis B.

We compared the effects of HBsAg-DC method and IFN-α or lamivudine method. The negative rate of HBeAg and the sera-reverse rate of HBeAg/anti-HBe of the two anti-virus methods were 30-40% and 20-30%[12-14], respectively. There was no significant difference. In our experiment, we treated two patients with HBsAg-DCs and lamivudine at the same time. At the first time follow-up, they lost HBeAg and had a decrease of 10^5 copies in HBV-DNA load. At the second time of follow-up, they were negative for HBV-DNA and one was positive for anti-HBe. They were stable in HBV-markers and had no fluctuation during 4 times of follow-up. It indicates that co-application of DC vaccine and other anti-virus drugs could enhance the response ability and have effects on rapid HBV elimination.

In conclusion, autologous HBsAg-DC vaccine can suppress virus replication, and reduce serum virus load and improve the reversion rate of HBeAg/anti-HBe. The treatment is simple with no side effect and the vaccine is very reliable. Thus, this DC treatment is one of the biological therapeutic methods with a good application prospect.

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