BALB/c小鼠腹腔接种HBV表面S抗原疫苗引起的CTL反应

Hepatitis B virus (HBV) is the major etiological agent responsible for chronic liver disease, and chronic HBV infection often leads to hepatic cirrhosis and hepatocellular carcinoma. At present, the number of patients with chronic HBV infection has reached approximately 350 million worldwide, and interferon and lamivudine prevail in the treatment. In spite of the high cost of interferon therapy, adverse effect is a frequent clinical entity while only roughly 35% of the patients may have HBe/anti-HBe seroconversion with negative HBV DNA. Lamivudine possesses stronger antiviral effect, but the high rate of relapse after withdrawal of a 12-month treatment course remains a problem, with as low as about 20% of the patients having long-term HBe/anti-HBe seroconversion with negative HBV DNA and genotypic resistance. Recent clinical and experimental studies have demonstrated the efficacy of immunization with HBV vaccine and specific anti-HBV cytotoxic T lymphocyte (CTL) transfer in elimination of HBV and fighting pre-existing infections[1-5], and in this study, we aim to test whether small surface antigen of HBV (S-HBsAg) may elicit CTL response in BALB/c mice and determine the dose of this vaccine that induce the strongest CTL repsonse.

MATERIALS AND METHODS

Materials

The recombinant hepatitis B (HB) vaccine, Recombivax HB, was a yeast-derived HBV small surface antigen produced by Merck & Co, containing L^d-restricted CTL epitope S_{28-39} (IPQSLOSWWTSL). The specific peptide IPQSLDSWWTSL (S_{28-39}), which represents a L^d-restricted CTL epitope of the S-HBsAg of H-2d BALB/c mice, was synthesized by Saibaisheng Co. and dissolved at the concentrations of 10-50 mg/ml in the stock solution containing 70% acetonitrile and 0.1% TFA or DMSO, and diluted with culture medium before use.

$H-2^d$ mastocytoma cell line P815 were purchased from Chinese Cell Culture Center.

Methods

Immunization of mice Female BALB/c($H-2^d$) mice of 6-10 weeks old were bred under standard pathogen- free conditions. The mice were grouped to receive intraperitoneal injections with Recombivax HB at different doses, with a booster injection 2 weeks later. Details of grouping and the doses injected are listed below:

Group 0 (n=10), in which the mice were injected with 5 μg adjuvant (aluminum

hydroxide); Group 0.65 (n=10), in which the mice received injection of 0.65 μg Recombivax HB; Group 1.25 (n=10), in which Recombivax HB was injected at the dose of 1.25 μg; Group 2.5 (n=10), in which Recombivax HB at the dose of 2.5 μg was used; Group 5 (n=10), in which the dose of Recombivax HB was 5 μg.

CTL assays

P815 cells (1×10^7) were treated with specific peptide and labeled with 100 μl $Na_2{}^{51}CrO_4$ for 1 h at 37 ℃, to serve as the target cells. The spleen cells as the effector cells were suspended in complete DMEM supplemented with 10% fetal calf serum (FCS), followed by treatment with 2-mercaptoenthanol (final concentration of 5×10^{-5} mol/L), murine interleukin (IL) -2 (final concentration of 5 U/ml). After 5 days of in vitro culture and washing twice, the cytotoxic effector lymphocytes were harvested and cocultured in triplicates with the target P815 cells at the ratio of 50∶1, with 1×10^4 cells in each well. After a 4-hour incubation at 37 ℃ and centrifugation, the supernant (100 μl) was collected for assessment of the radioactivity with γ-counter.

Two weeks after the primary inoculation, the spleen lymphocytes isolated from half of the mice were incubated with ^{51}Cr-labeled P815 cells, and the percentage of specific ^{51}Cr release (PSR) of the spleen lymphocytes for only the primary immunization was calculated; four weeks after the booster immunization, the spleen lymphocytes isolated from the rest of the mice were incubated in the presence or absence of ^{51}Cr-labelled P815 cells, and PSR of the spleen lymphocytes was calculated.

PSR was calculated by (experimental release - spontaneous release)/(maximum release - spontaneous release)×100%, where the experimental release was defined as the mean cpm released by the target cells in the presence of effector cells. Maximum release was the mean cpm measured after lysis of the target cells with 5% Triton X-100. Spontaneous release was the mean cpm present in the culture medium of the target cells alone. The purpose of calculating PSR was to eliminate the possible interference of the background factors.

Statistical analysis

A non-parametric Mann-Whitney U test was used to compare the results between different groups. A P value of less than 0.05 or 0.01 was considered statistically significant or very statistically significant, respectively.

RESULTS

In the CTL assay, the ratio of spontaneous release to maximum release was 28.76%±13.45% for the target cells.

In BALB/c mice with booster immunization, the PSR of the spleen lymphocytes of the mice in Groups 0, 0.65, 1.25, 2.5 and 5 was 31.21%±9.23%, 42.36%±19.32%, 91.21%±22.97%, 69.25%±24.13%, and 51.49%±21.661% respectively, which was the highest in Group 1.25 and lowest in Group 0. PSR was the lowest in Group 0.65 among the mice with booster immunization.

In mice with only primary immunization, PSR of the spleen lymphocytes in Groups 0, 0.65, 1.25, 2.5, and 5 was 27.34%±14.25%, 32.27%±15.35%, 56.28%±24.35%, 44.34%±18.65% and 40.76±56% respectively.

For the target cells without treatment with the specific peptide, the PSR of the spleen lymphocytes from the mice with booster immunization were 20.56%±17.28%, 22.37%±18.29%, 34.29%±21.23%, 21.27%±20.30%, 32.38%±16.56%, respectively.

DISCUSSION

HBV is a noncytopathic DNA virus, and adult onset of HBV infection usually results in self-limited acute hepatitis followed by viral clearance, with only 5% of the infected adults developing chronic infection. In patients with self-limited acute HBV infection, the virus can be spontaneously cleared from the serum, accompanied by increased cellular immune responses, especially CTL response. In the majority of the patients with chronic hepatitis, HBV-specific CTL response was thought to be low so as to give rise to HBV persistence. The therapy of HBV infection depends mainly on host cellular immune response. CTL play important roles in the elimination of the virus-infected cells, and in HBV-infected patients, CTL is involved in the reduction of viral load. Although immunization with S-HBsAg vaccine has been acknowledged to induce humoral immune response and protect against HBV attack, the questions weather this vaccine also elicits cellular immune response, and if so, what the optimal dose is to induce the strongest CTL response, have not been answered[1][2][3][4].

To investigate cell-mediated immune responses to S-HBsAg, in vivo primed spleen cells from immunized mice were stimulated in vitro with a specific peptide and tested for CTL activity against P815 cells treated with the specific peptide. We found that the PSR in the vaccinated mice at the doses of 0.65, 1.25, 2.5, and 5 μg was all significantly higher than that in the control group without vaccination, and also higher than those in the parallel groups with the target cells not treated with the specific peptide, indicating specific CTL response to S-HBsAg vaccination. In the experimental groups, PSR tended to increase as the dose of vaccination increased from 0.65 to 1.25 μg, but not with the doses from 2.5 to 5 μg. PSR in Group 1.25 was significantly higher than those in other groups ($P<0.05$). The difference in PSR between Groups 0.65 and 5 was not significant ($P>0.05$), suggesting that the CTL response is dependent on the dose of immunization. The PSR in mice with booster immunization was significantly higher than that in the mice with only primary inoculation, indicating that the booster immunization enhanced the CTL response.

Specific CTLs are the major cells participating in the cellular immune response against acute, chronic, and persistent infections with intracellular pathogens such as viruses, and function as a crucial defense against the spread of these pathogens through both cytolysis of infected cells they recognized and noncytolytic mechanisms such as cytokine production (γ-interferon and tumor necrosis factor α). CTLs specific for the epitopes in HBV contribute to the control of HBV infection, which were readily detectable in patients with HBV clearance (self-limited acute hepatitis recovering from acute HBV infection exhibits a polyclonal and multispecific CTL response to HBsAg) but not in cases of chronic infection. It is now clear that therapeutic vaccination with immunogenic peptides depends heavily on CTL induction. Vaccination strategies favoring the induction of stable cell-mediated responses might protect against subsequent viral infections. Patients with HBV infection benefit from injection of S-HBsAg through induction of viral

clearance by enhancement of suboptimal cellular immune responses. Injection of this vaccine induces marked cellular immune responses to HBsAg, significantly decreasing HBV viral load and increasing the rate of HBe/anti-HBe seroconversion. The immunotherapeutic effect of the vaccination in chronically HBV-infected patients to enhance HBV-specific cellular immune responses may be of value in the control of persistent infection.

CTLs recognize the target cells though the CTL epitope and the major histocompatibility complex-Ⅱ determinants on the surface of the target cells. In this study, the S-HBsAg vaccine contained HBV Ld-restricted CTL epitope S_{28-39} (IPQSLOSWWTSL). P815 cells treated with the specific peptide expressed H-2^d MHC-II restricted determinants similar with the BALB/c. The in vivo primed spleen cells from the immunized BALB/c(H-2^d) mice stimulated in vitro with specific peptide exhibited specific cytotoxic reactivity against P815 cells, and high-dose vaccination resulted in substantial specific CTL activity of the spleen cells, which was markedly enhanced by a booster vaccination[5][6][7][8][9][10][11].

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