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Proteomic analysis of augmented immune responses in mouse by prime-and-boost immunization strategy with DNA vaccine coding HBsAg and rHBsAg protein

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Abstract

Prime-and-boost vaccination strategy with DNA and protein vaccines is broadly adopted to augment the immunogenicity of both vaccines, but the mechanism is ambiguous. Antigen-specific immunological memories in humoral and cellular immune responses were examined in mice after immunization with different regimens, by the evaluation of persistence of antibody production and CTL activity, as well as T cell proliferation assay. Stronger immunological memories were demonstrated in group DDS (mice immunized with rHBsAg after twice DNA priming), well associated with the induced higher level of antibody and CTL activity. Comparative serum proteomics was employed to investigate the possible mechanisms of immunopotentiation effects. In comparison with proteome of non-vaccinated mice, 5 proteins in group DDS were up-regulated and 17 proteins down-regulated by more than 2.5-fold in quantity, whereas in group SSS (mice immunized with rHBsAg three times) 7 up-regulated and 10 down-regulated. Periplakin, F-box protein 30 and calpain detected only in group DDS have been approved to contribute to the immunopotentiation effect by this vaccination regime, which might be established as an surrogate marker of successful vaccination and provides research target for molecular mechanism of vaccinology.

Keywords: Hepatitis B virus; DNA vaccine; Prime-and-boost; Proteomics

1. Introduction

Hepatitis B poses a serious worldwide problem. Chronically infected patients with active liver disease carry a high risk of developing cirrhosis and hepatocellular carcinoma [1]. Lack of satisfactory antiviral treatment against HBV makes the development of an efficient vaccine eagerly desirable. Though current recombinant subunit vaccines have been widely used by inducing effective humoral immunity [2], the major disadvantage of these vaccines is the disability to induce cellular immunity, which is essential for the prophylaxis and therapy of the disease.

DNA vaccine encoding HBV proteins has been adequately documented to elicit strong and durable humoral and cell-mediated immune responses including the secretion of cytokines and formation of cytotoxic T lymphocytes (CTL) in mice [3], chimpanzees [4] and humans [5]. But some phase-1 clinical trials of DNA vaccines have demonstrated that the magnitude of immune responses induced in humans is generally lower than that in small animals, and the amount of DNA required for effective immunization is much larger [4,5]. The application of DNA vaccines has been demonstrated to be relatively safe with the risk of genetic mutation due to plasmid DNA negligible. However, the immunogencity of DNA vaccine must be elevated to reduce the required dose [6]. Therefore, the immunogenicity of DNA vaccines must be elevated to reduce the required dosage and the risk of integration.

Abbreviations: HBV, hepatitis B virus; rHBsAg, recombinant hepatitis B surface antigen; 2DE, two-dimensional electrophoresis; MALDI–TOF MS, matrix-assisted laser desorption ionization– time-of-flight mass spectrometry.

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A vaccination strategy of DNA priming followed by protein or viral vector boosting has been widely reported to greatly improve the effect of these DNA-based vaccines against HIV [7], tuberculosis [8] and malaria [9]. Recently, our group has firstly adopted a series of combinatorial regimens involving different times, orders and protocols of vaccination with DNA and/or subunit vaccines, indicating that consecutive DNA priming followed by boosting with recombinant hepatitis B surface antigen (rHBsAg) dramatically improves the efficacy of the DNA vaccine [10]. However, the mechanism of the immunopotentiation effects elicited by prime-and-boost strategy remains unsolved.

The induction of immunological memory following vaccination is critical for a successful vaccine. It was well documented that a small fraction of naïve T cells develop into memory T cells after antigen stimulation. The superior efficacy of memory T cells is a consequence of their increased frequency, their ease in activation, and their priority to reside within or migrate into peripheral tissues [11]. With these privileges, memory T cells play an important role in secondary immune responses and thus may relevantly be considered a possible explanation for the immunopotentiation effect elicited by prime-and-boost strategy. In our current study, the proliferation of T cells in mice vaccinated with different regimens revealed augmented immune responses were associated with relatively stronger immunological memory in mice immunized twice with DNA followed by a single rHBsAg boost.

Proteomics represents an exciting high-throughput new way to examine large-scale proteins simultaneously and is already leading to improved comprehension of many physiological and pathological conditions at molecular level [12,13]. The use of two-dimensional electrophoresis (2DE) was used to separate serum proteins from mice immunized by different vaccination regimens, obtaining six specifically expressed proteins which were identified by matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI–TOF MS). Of the six proteins, F-box only protein 30, periplakin, and calpain 11 expressed only in the group immunized by rHBsAg after twice DNA priming show some characteristics favorable to antigen capturing, processing and presentation. The application of powerful proteomic methods on vaccine research not only gives us further understanding on prime-and-boost vaccination strategy, but also provides us a promising approach to investigate the molecular mechanisms of vaccinology.

2. Materials and methods

2.1. DNA vaccine preparation

The plasmid pVAX-S encoding HBV small envelope protein was constructed, amplified and purified as described in our previous work [10].

Table 1
Prime-and-boost vaccination regimen of four groups of C57BL/6 mice

Group	Week 0	Week 4	Week 8
DDD	pVAX-S	pVAX-S	pVAX-S
DDS	pVAX-S	pVAX-S	rHBsAg
SSD	rHBsAg	rHBsAg	pVAX-S
SSS	rHBsAg	rHBsAg	rHBsAg

2.2. Mice

All the C57BL/6 mice were bred and humanely cared under SPF condition in the Laboratory Animal Center of the Second Military Medical University. Female mice (20 per group) 8 weeks old were chosen at the start of three-round vaccination.

2.3. DNA and protein vaccination

DNA and protein vaccination were carried out as described in ref. [10]. For DNA vaccination, intramuscular injection of 100 μg pVAX-S was performed in quadriceps. For protein vaccination, mice were injected subcutaneously in the lower back midline with 0.8 μg of purified rHBsAg. Four groups of C57BL/6 mice were immunized three rounds with 4 weeks interval by different regimens shown in Table 1. For memory T cell preparation dozens of 8 weeks old female mice were immunized once by rHBsAg with the same dose.

2.4. Specific antibody analysis

Sera from the immunized mice were collected by tail bleeding at selected time points. The total HBsAg-specific antibody was detected by ELISA (Fosun LongMarch; Shanghai, China).

2.5. Preparation of BMDCs

DC were generated from bone marrow progenitors as described by Lutz et al. [14]. Briefly, freshly prepared bone marrow cells were cultured in Click RPMI 1640 medium supplemented with 10% heat-inactivated FCS, 2 mM L-glutamine, 10 mM HEPES buffer, 60 μg/ml penicillin, and 20 μg/ml gentamicin in the presence of 200 U/ml GM-CSF (PeproTech, London, U.K.). Cultures were fed with GM-CSF on days 3, 6, and 8. After 10 days, the nonadherent cells were collected and identified by flow cytometry (FACSCaliburTM; BD Biosciences; San Jose, USA) as $CD83^{+}CD86^{+}$ cells.

2.6. T lymphocyte separation and identification

Spleens were removed aseptically and ground on a 200-mesh stainless steel sieve in medium RPMI-1640. Mononuclear cells (mostly splenic lymphocytes) were obtained by density gradient centrifugation applying Ficoll-Hypaque (1.077 g/ml; HaoYang Biological Manufacture, Tianjin, China) and incubated in a 10-ml syringe filled with