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• VIRAL HEPATITIS •

HBV DNA vaccine with adjuvant cytokines induced specific immune responses against HBV infection

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Abstract

AIM: To seek for an effective method to improve the immune responses induced by DNA vaccine expressing HBV surface antigen (pCR3.1-S) in Balb/c mice (H-2^d).

METHODS: The pCR3.1-S plasmid and the eukaryotic expression vectors expressing murine IL-2 (pDOR-IL-2) or IL-12 (pWRG3169) were injected into mice subcutaneously. The immune responses to pCR3.1-S and the adjuvant effect of the cytokines plasmid were studied. Meanwhile the effect of pCR3.1-S on anti-translated subcutaneous tumor of P815 mastocytoma cells stably expressing HBsAg (P815-HBV-S) was also studied. Anti-HBs in serum was detected by enzyme-linked immunoadsordent assay (ELISA) and HBsAg specific cytotoxic T lymphocytes (CTLs) activity was measured by ^{51}Cr release assay. After three weeks of DNA immunization, the cells of P815-HBV-S were inoculated into mice subcutaneously and the tumor growth was measured every five days. The survival rate and living periods of mice were also calculated.

RESULTS: After 8 wk DNA immunization, the *A* 450 nm values of sera in mice immunized with pCR3.1, pCR3.1-S and pCR3.1-S codeliveried with IL-2 or IL-12 plasmids were 0.03±0.01, 1.24±0.10, 1.98±0.17 and 1.67±0.12 respectively. Data in mice codeliveried pCR3.1-S with IL-2 or IL-12 plasmids were significantly higher than that of mice injected pCR3.1 or pCR3.1-S only. The HBsAg specific CTL activities in mice coinjected with pCR3.1-S and IL-2 or IL-12 eukaryotic expression vectors were (61.9±7.1) % and (73.3±8.8) % , which were significantly higher than that of mice injected with pCR3.1 (10.1±2.1) % or pCR3.1-S (50.5 ±6.4) %. The HBsAg specific CTL activities in mice injected with pCR3.1, pCR3.1-S, pCR3.1-S combined with IL-2 or IL-12 eukaryotic expression vectors decreased significantly to (3.2±0.8) %, (10.6±1.4) %, (13.6±1.3) % and (16.9±2.3) % respectively after the spleen cells were treated by anti-$CD8^+$ monoclonal antibody, but presented no significant change to anti-$CD4^+$ monoclonal antibody or unrelated to monoclonal antibody. The HBV-S DNA vaccine (pCR3.1-S) could evidently inhibit the tumor growth, prolong the survival period of mice and improve the survival rate of mice and these effects could be improved by IL-12 gene codeliveried.

CONCLUSION: HBV DNA vaccine has a strong antigenicity in humoral and cellular immunities, which can be promoted by plasmid expressing IL-2 or IL-12. CD8+ cells executed the CTL activities. DNA vaccine may be useful for both prophylaxis and treatment of HBV infection.

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INTRODUCTION

Many animal models of infectious diseases have been reported[1-3] which shows DNA vaccine induced broad range of protective immunities, including antibodies, $CD8^+$CTL, $CD4^+$Th cells against challenge with the pathogens, such as plasmodium[4], influenza virus[5] simplex virus[6] and HIV-1[7]. Application of this genetic vaccination approach has been extended to the treatment of cancers[8,9] as well as allergic diseases[10,11] and autoimmune disease[12]. Because DNA vaccines can induce weak and short-lived immune responses in large out-bred animal[13], seeking for an effective way to promote the immune responses of DNA immunization is an urgent case. Relying on the knowledge above, we constructed a recombination vector expressing HBV S protein, pCR3.1-S, to study the possibility of DNA vaccine in controlling and preventing the HBV infection. In addition, the eukaryotic expression vectors expressing murine IL-2 or IL-12 were coimmunized to mice with pCR3.1-S and their effects as adjuvants for immune responses were also studied. Meanwhile, we established the HBV-infectious animal model through the inoculating of the P815-HBV-S and observed the treatment and preventive effect of pCR3.1-S to HBV infection *in vivo*.

MATERIALS AND METHODS

Plasmids, cell lines and mice

Plasmid expressing hepatitis B virus surface antigen (pCR3.1-S)[14] was constructed by Prof. Yao ZHQ (in this Department). Plasmids expressing murine IL-2 (pDOR-IL-2) and IL-12 (pRW1369)[15] were generous gift from Dr. Feng ZHH(in this Department). P815 mastocytoma cells were generous gift from Dr. Zhao (Department of Pathology, the Fourth Military Medical University). Female Balb/c (H-2^d) mice were obtained from the Center for Experimental Animals of the Fourth Military Medical University and used at the age of 5-8 weeks.

Transfection and expression of pCR3.1-S in P815

P815 cells were maintained in RPMI 1640 (Sigma) with 10 mL· L^{-1} fetal bovine serum in a six-well tissue culture plate at 37 ℃ in 5 % CO_2 humidified atmosphere and then transfected with the pCR3.1-S or the pCR3.1 alone by using lipofectamine (GIBCO). For each transfection 20 μg of plasmid and 15 μL of lipofectamine in 0.2 mL of serum free medium were mixed in tube for 30 minutes. After 0.8 mL of serum-free medium were added to the tube, the DNA-lipofectamine complexes were overlaid onto the cells. After incubated 12 hours, the cells were washed two times with the complete culture medium and

the medium was replaced with 2 mL of the complete culture medium. After another 24-48-hour incubation, the cells were transfered from the culture medium,which was then replaced by medium contained 300 mg· L^{-1} G_{418} (Promega). Two weeks later, the G_{418}-resistant clones were selected and the expression of HBsAg was detected by using of indirect immunofluorescence (IIF). The HBsAg expressed cells were designated as P815-HBV-S and used as the target cells for CTL assay.

DNA immunization in mice

Four groups of mice were used, each consisting of 5 mice which were immunized with one of the following regiments in 100 μL of sterile saline: (1) 100 μg of pCR3.1-S; (2) mixture of 100 μg of pCR3.1-S and 100 μg of pDOR-IL-2 (IL-2); (3) mixture of 100 μg of pCR3.1-S and 100 μg of pRW1369 (IL-12); (4) 100 μg of pCR3.1 vector. The mice in the last group served as negative control. All injections were done intramuscularly into the lift thigh quadriceps muscle of mice at 0, 2, 4, 6 and 8 weeks.

HBsAg-specific antibody assay

Sera samples were collected by tail bleeding at different times, beginning at 1 wk after immunization, and the presence of HBsAg-specific antibody was analyzed by ELISA. The ELISA kits for the HBsAg-specific antibody detection were purchased from Huamei Co. and performed according to the manufacturer' s instructions.

CTL assay

Spleen cells of mice were segregated 8 weeks after immunization and the CTL activities were measured by ^{51}Cr releases assay. $Na^{51}CrO_4$ was purchased from Dubang Co. Target cells (1×10^6) were labeled with 3.7MBq radiolabeled sodium chromate. The assays were performed in triplicate with 1×10^5 targets/well at various effector cell/target cell (E:T) ratios of 100:1. Results were expressed according to the formula: % specific lysis= (experimental release-spontaneous release)/(maximum release-spontaneous release). Experimental release represents the mean count per-minute released by target cells in the presence of effector cells. Maximum release represents the radioactivity released after lysis of target cells with 50 g· L^{-1} Triton X-100. Spontaneous release represents the radioactivity present in medium derived from target cells alone.

Blocking of CTL response by monoclonal antibodies

At the effector cell/target cell ratio of 100:1, CTL assays were performed in the presence of 10 mg· L^{-1} of anti-$CD4^+$ or anti-$CD8^+$ monoclonal antibody added to the spleen cells in 96-well plates. As a control an unrelated antibody was added to the spleen cells. The monoclonal antibodies to mouse CD4+ or CD8+ cell were purchased from Sigma Chemical Co.

DNA vaccine against subcutaneous translated tumor

Four groups of mice were used, each consisting of 5 mice immunized with one of the following regiments in 100 μL of sterile saline: (1) 100 μg of pCR3.1-S;(2) mixture of 100 μg of pCR3.1-S and 100 μg of pRW1369 (IL-12). (3) 100 μg of pCR3.1; one group of mice without immunization. Three weeks after DNA immunization,cells of P815-HBV-S were inoculated into mice by subcutaneous injection in abdomen. The growing tumors were measured every five days with a calipers using average diameter. The survival period of mice was observed and the survival rate of mice was calculated.

Statistical analysis

Data were reported as $\bar{x}\pm s$ and were analyzed by professional statistical computer software SPSS. Significance was set at $P<0.05$.

RESULTS

Codelivery of cytokines gene augmented the titer of antibody induced by pCR3.1-S

The pCR3.1-S showed a strong antigenicity in humoral immunity and the anti-HBsAg could be detected in sera of mice after pCR3.1-S vaccination. The serum titres of anti-HBsAg in mice increased with the times of immunization in a period of time. The titres of anti-HBsAg in sera of mice were significantly promoted by genes expressed murine IL-2 or IL-12, especially by IL-2 gene (Figure 1).

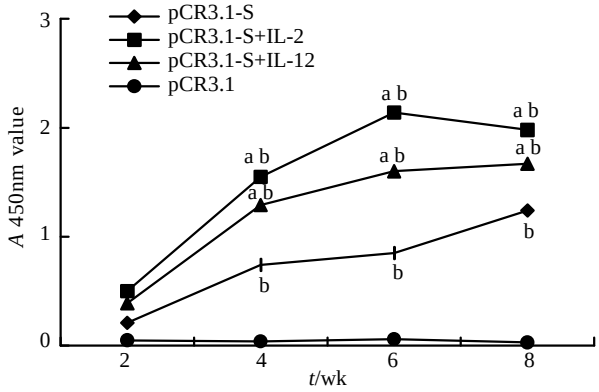

Figure 1 Serum anti-HBsAg level of Balb/c mice. $^bP<0.01$ *vs* pCR3.1; $^aP<0.05$ *vs* pCR3.1-S

Cytokines gene effected on the CTL activity induced by pCR3.1-S

The HBsAg specific CTL activities were developed in the mice after pCR3.1-S immunization. The CTL activities were augmented by coimmunized with IL-2 or IL-12 gene. The mice immunized with pCR3.1 alone did not elicited detectable HBsAg specific CTL activities. IL-12 was more effective than IL-2 in promoting the HBsAg specific CTL activities. The CTL activity was blocked by anti-$CD8^+$ monoclonal antibody but not by anti-$CD4^+$ monoclonal antibody or unrelated antibody (Table 1). Taken together, these results indicated that the CTL activity induced by pCR3.1-S *in vivo* was executed by cells expressing $CD8^+/CD4^-$ surface phenotype and the CTL activity could be enhanced or suppressed depending on the cytokines gene expressed.

Table 1 The effect of cytokines gene on CTL activity induced by pCR3.1-S (n=5; $\bar{x}\pm s$ %)

Group	Untreated	mAb		
		Unrelated	Anti-$CD4^+$	Anti-$CD8^{+b}$
pCR3.1	10.1±2.1	10.7±1.9	9.7±1.2	3.2±0.8
pCR3.1-S	50.5±6.4	49.7±6.1	48.3±5.9	10.6±1.4
pCR3.1-S+IL-2	61.9±7.1	62.0±6.8	56.2±7.5	13.5±1.9
pCR3.1-S+IL-12	73.3±8.8	69.9±7.6	75.6±9.1	16.9±2.3

$^bP<0.01$ *vs* unrelated mAb or CD_4^+ mAb

DNA vaccine inhibits the formation of subcutaneous translating tumor derived from P815-HBV-S

After inoculated with P815-HBV-S, all five mice with or without pCR3.1 (100 %) formed the tumor. The rate of tumor formation was 20 % (1/5) in mice immunized with pCR3.1-S and there was no tumor formed in mice coimmunized with pCR3.1-S and IL-12. The survival rate of mice immunized with pCR3.1-S along or coimmunized with IL-12 increased significantly (Figure 2) and the tumor growth was evidently slower than that of mice immunized with or without pCR3.1 (Figure 3).

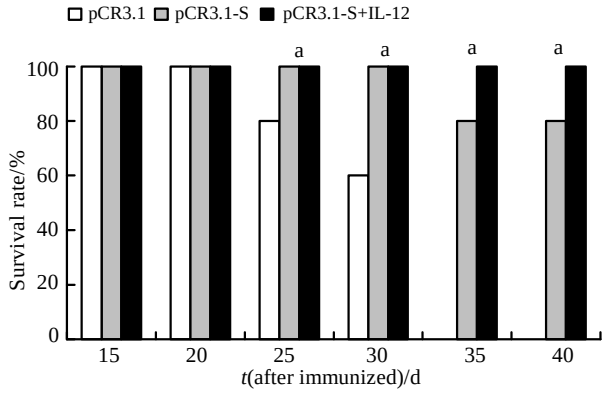

Figure 2 Survival rate in different group. $^{a}P<0.05$ *vs* pCR3.1

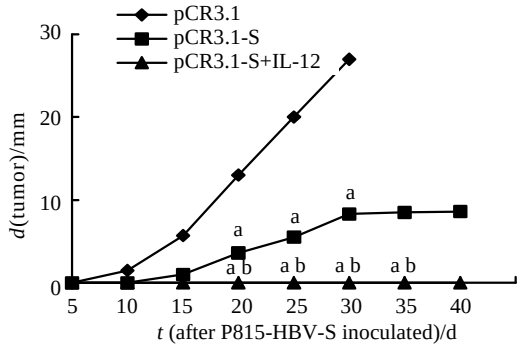

Figure 3 Growth curve of subcutaneous translating tumor in different group of mice. $^{b}P<0.01$ *vs* pCR3.1; $^{a}P<0.05$ *vs* pCR3.1-S

DISCUSSION

HBV infection is very common in China[16-23]. It is estimated that approximately 130 million people in the world is infected by hepatitis B virus (HBV). These people are at risk of developing chronic hepatitis leading to liver cirrhosis and hepatocellular carcinoma. Up to now, vaccination is a main way in prevention[24-31].

It has been suggested that the MHC class II and I restricted T cell responses to the virus are relatively weak during chronic HBV infection[28] and there are no specific therapies to cope with it. DNA vaccine contains the gene for an antigenic portion of a pathogen, such as the core or the envelope protein, usually under the transcriptional control of a viral promoter[29,30]. In DNA-based vaccination, immunogenic proteins are expressed in transfected cells of the vaccine recipients in their native conformation with correct post-translational modifications from antigen-encoding expression plasmid DNA *in vivo*. This ensures the integrity of antibody-defined epitopes and supports the generation of protective antibody. DNA vaccination is furthermore an exceptionally potent strategy to stimulate CD8+ cytotoxic T lymphocyte (CTL) responses because antigenic peptides are efficiently generated by endogenous processing of intracellular protein antigen[31].

The results of our experiment indicate that the plasmids expressing either IL-2 or IL-12 can enhance the specific humoral and cellular immune responses against HBV infection elicited by pCR3.1-S in mice. The former mainly enhances the level of the HBsAg specific antibody and the latter mainly enhances the HBsAg specific CTL activity.

IL-2 can enhance the immune responses through promoting proliferation and differentiation of B cells and the antibody development. The proliferation and activation of many kinds of T-cells and the production of various cytokins can also be promoted and stimulated by IL-2. The effect of IL-2 enhancing the level of antibody may be related to its ability to induce the increase of the Th1 cells but not Th2 cells. This can increase IgG2a type of antibody and lead to the increase of the total level of the antibody subsequently[32-34]. IL-12 is so far the most potent cytokine with the widest scope of modulation of immune responses. The immune responses can be modulated by IL-12 promoting the production of Th1 type T cells and secretion of other cytokines, stimulating the polarization and proliferation of T cells and through promoting the maturity of CTL cells and LAK cells. All of these can enhance the ability of the host to kill and eliminate pathogens. The adjuvanticitities of cytokines were also observed by others[25,32-36].

The situations *in vivo* are different from that *in vitro* after all. In order to search the preventive and therapeutical effects of HBV DNA vaccine to the HBV infection *in vivo*, we serve the mouse injected with P815-HBV-S cells subcutaneously for an animal model of HBV infection. The preventive and therapeutical effects of pCR3.1-S on HBV infectious animal model were investigated by observing the inhibiting effects of pCR3.1-S on the neoplasia of P815-HBV-S cells inoculated by subcutaneous injection. The result indicates that the pCR3. 1-S can reduce the formative rate of the subcutaneous translating tumor significantly, inhibit the growth of tumor, prolong the living periods and promote the survival rate of mice injected with the P815-HBV-S cells. Maybe all of these relate to the specific killing effect of CTLs to P815-HBV-S cells induced by DNA vaccine. Furthermore, these effects of pCR3.1-S can be enhanced obviously by IL-12 gene as shown in the experiment. The therapeutic potential of DNA-based immunization for the chronic HBV carrier states has also been demonstrated in a transgenic mouse model[37]. This mode of immunization has been shown to successfully eliminate HBsAg in circulation.

These features of DNA-based immunization make it an attractive strategy for prophylactic and therapeutic vaccination against extra- and intracellular pathogens. Recently, DNA vaccines induced the specific humoral[38] and cellular[39-41] immune responses were observed in human experiments. The results of these experiments suggest that DNA vaccine might be a potential therapy for chronic HBV infection.

REFERENCES

1 **Loirat D**, Lemonnier FA, Michel ML. Multiepitopic HLA-A*0201-restricted immune response against hepatitis B surface antigen after DNA-based immunization. *J Immunol* 2000; **165**: 4748-4755
2 **Dunham SP**, Flynn JN, Rigby MA, Macdonald J, Bruce J, Cannon C, Golder MC, Hanlon L, Harbour DA, Mackay NA, Spibey N, Jarrett O, Neil JC. Protection against feline immunodeficiency virus using replication defective proviral DNA vaccines with feline interleukin-12 and -18. *Vaccine* 2002; **20**: 1483-1496
3 **Huang ZH**, Zhuang H, Lu S, Guo RH, Xu GM, Cai J, Zhu WF. Humoral and cellular immunogenecity of DNA vaccine based on hepatitis B core gene in rhesus monkeys. *World J Gastroenterol* 2001; **7**: 102-106
4 **Le TP**, Coonan KM, Hedstrom RC, Charoenvit Y, Sedegah M, Epstein JE, Kumar S, Wang R, Doolan DL, Maguire JD, Parker SE, Hobart P, Norman J, Hoffman SL. Safety, tolerability and humarol immune responses after intramuscular administration of a malaria DNA vaccine to healthy adult volunteers. *Vaccine* 2000; **18**: 1893-1901
5 **Cox RJ**, Mykkeltvedt E, Robertson J, Haaheim LR. Non-lethal viral challenge of influenza haemagglutinin and nucleoprotein DNA vaccinated mice results in reduced viral replication. *Scand J Immunol* 2002; **55**: 14-23
6 **Strasser JE**, Arnold RL, Pachuk C, Higgins TJ, Bernstein DI. Herpes simplex virus DNA vaccine efficacy: effect of glycoprotein D plasmid constructs. *J Infect Dis* 2000; **182**: 1304-1310
7 **Fuller DH**, Rajakumar PA, Wilson LA, Trichel AM, Fuller JT, Shipley T, Wu MS, Weis K, Rinaldo CR, Haynes JR, Murphey-Corb M. Induction of mucosal protection against primary, heterologous simian immunodeficiency virus by a DNA vaccine. *J Virol*

2002; **76**: 3309-3017
8 **Gavarasana S**, Kalasapudi R S, Rao T D, Thirumala S. Prevention of carcinoma of cervix with human papillomavirus vaccine. *Indian J Cancer* 2000; **37**: 57-66
9 **Thirdborough SM**, Radcliffe JN, Friedmann PS, Stevenson FK. Vaccination with DNA encoding a single-chain tcr fusion protein induces anticlonotypic immunity and protects against t-cell lymphoma. *Cancer Res* 2002; **62**: 1757-1760
10 **Horner AA**, Van Uden JH, Zubeldia JM, Broide D, Raz E. DNA-based immunotherapeutics for the treatment of allergic disease. *Immunol Rev* 2001; **179**: 102-118
11 **Lee YL**, Ye YL, Yu CI, Wu YL, Lai YL, Ku PH, Hong RL, Chiang BL. Construction of single-chain interleukin-12 DNA plasmid to treat airway hyperresponsiveness in an animal model of asthma. *Hum Gene Ther* 2001; **12**: 2065-2079
12 **Garren H**, Ruiz PJ, Watkins TA, Fontoura P, Nguyen LT, Estline ER, Hirschberg DL, Steinman L. Combination of gene delivery and DNA vaccination to protect from and reverse Th1 autoimmune disease via deviation to the Th2 pathway. *Immunity* 2001; **15**: 15-22
13 **Chaplin PJ,** De Rose R, Boyle JS, McWaters P, Kelly J, Tennent JM, Lew AM, Scheerlinck JP. Targeting improves the efficacy of a DNA vaccine against Corynebacterium pseudotuberculosis in sheep. *Infect Immun* 1999; **67**: 6434-6438
14 **Li WB**, Yao ZQ, Zhou YY, Feng ZH. Studies on immunization with HBV gene vaccine plus HBsAg protein in mice. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 188-190
15 **Rakhmilevich AL**, Turner J, Ford MJ, McCabe D, Sun WH, Sondel PM, Grota K, Yang NS. Gene gun-mediated skin transfection with interleukin 12 gene results in regression of established primary and metastatic murine tumors. *Proc Natl Acad Sci USA* 1996; **93**: 6291-6296
16 **Guan XJ**, Wu YZ, Jia ZC, Shi TD, Tang Y. Construction and characterization of an experimental ISCOMS-based hepatitis B polypeptide vaccine. *World J Gastroenterol* 2002; **8**: 294-297
17 **Chen XS**, Wang GJ, Cai X, Yu HY, Hu YP. Inhibition of hepatitis B virus by oxymatrine *in vivo*. *World J Gastroenterol* 2001; **7**: 49-52
18 **Huang ZH**, Zhuang H, Lu S, Guo RH, Xu GM, Cai J, Zhu WF. Humoral and cellular immunogenecity of DNA vaccine based on hepatitis B core gene in rhesus monkeys. *World J Gastroenterol* 2001; **7**: 102-106
19 **Fang JN**, Jin CJ, Cui LH, Quan ZY, Choi BY, Ki MR, Park HB. A comparative study on serologic profiles of virus hepatitis B. *World J Gastroenterol* 2001; **7**: 107-110
20 **Guo SP**, Wang WL, Zhai YQ, Zhao YL. Expression of nuclear factor-κB in hepatocellular carcinoma and its relation with the X protein of hepatitis B virus. *World J Gastroenterol* 2001; **7**: 340-344
21 **You J**, Zhuang L, Tang BZ, Yang H, Yang WB, Li W, Zhang HL, Zhang YM, Zhang L, Yan SM. Interferon alpha with Thymopeptide in the treatment of chronic hepatitis B. *Shijie Huaren Xiaohua Zazhi* 2001; **9**: 388-391
22 **He XS**, Huang JF, Chen GH, Fu Q, Zhu XF, Lu MQ, Wang GD, Guan XD. Orthotopic liver transplantation for fulminant hepatitis B. *World J Gastroenterol* 2000; **6**: 398-399
23 **Zhao LS**, Qin S,Zhou TY, Tang H, Liu L, Lei BJ. DNA-based vaccination induces humoral and cellular immune responses against hepatitis B virus surface antigen in mice without activation of C-myc. *World J Gastroenterol* 2000; **6**: 239-243
24 **Du DW**, Zhou YX, Feng ZH, Li GY, Yao ZQ. Study on immunization of anti-subcutaneous transplanting tumor induced by gene vaccine. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 955-957
25 **Du DW**, Zhou YX, Feng ZH, Yao ZQ, Li GY. Immune responses to interleukin 12 and hepatitis B gene vaccine in H^2-d mice. *Shijie Huaren Xiaohua Zazhi* 2000; **8**: 128-130
26 **Liu HB**, Meng ZD, Ma JC, Han CQ, Zhang YL, Xing ZC, Zhang YW, Liu YZ, Cao HL. A 12-year cohort study on the efficacy of plasma-derived hepatitis B vaccine in rural newborns. *World J Gastroenterol* 2000; **6**: 381-383
27 **Li H**, Wang L, Wang SS, Gong J, Zeng XJ, Li RC, Nong Y, Huang YK, Chen XR, Huang ZN. Research on optimal immunization strategies for hepatitis B in different endemic areas in China. *World J Gastroenterol* 2000; **6**: 392-394
28 **Geissler M**, Tokushige K, Wakita T, Zurawski VR Jr, Wands JR. Differential cellular and humoral immunization using chimeric constructs. *Vaccine* 1998; **16**: 857-867
29 **Reyes-Sandoval A**, Ertl H C. DNA vaccines. *Curr Mol Med* 2001; **1**: 217-243
30 **Kwissa M**, Unsinger J, Schirmbeck R, Hauser H, Reimann J. Polyvalent DNA vaccines with bidirectional promoters. *J Mol Med* 2000; **78**: 495-506
31 **Schirmbeck R**, Reimann J. Revealing the potential of DNA-based vaccination: lessons learned from the hepatitis B virus surface antigen. *Biol Chem* 2001; **382**: 543-452
32 **Du DW**, Liu QQ, Chen HM, Li JG, Lian JQ, Feng ZH, Zhou YX, Yao ZQ. Immune adjuvant effect of eukaryotic expression vetor with Interleukin-2 on hepatitis B gene vaccine. *Shanghai Mianyixue Zazhi* 2001; **21**: 77-79
33 **Geissler M**, Bruss V, Michalak S, Hockenjos B, Ortmann D, Offensperger WB, Wands JR, Blum HE. Intracellular retention of hepatitis B virus surface proteins reduces interleukin-2 augmentation after genetic immunizations. *J Virol* 1999; **73**: 4284-4292
34 **Li WB**, Yao ZQ, Zhou YX, Feng ZH. Effect of interleukin-2 on the potency of genetic vaccines of hepatitis B virus. *Di-si Junyi Daxue Xuebao* 1999; **20**: 747-749
35 **Scheerlinck JP**, Casey G, McWaters P, Kelly J, Woollard D, Lightowlers MW, Tennent JM, Chaplin PJ. The immune response to a DNA vaccine can be modulated by co-delivery of cytokines gene using a DNA prime-protein boost strategy. *Vaccine* 2001; **19**: 4053-4060
36 **Noormohammadi AH**, Hochrein H, Curtis JM, Baldwin TM, Handman E. Paradoxical effects of IL-12 in leishmaniasis in the presence and absence of vaccinating antigen. *Vaccine* 2001; **19**: 4043-4052
37 **Oka Y**, Akbar SM, Horiike N, Joko K, Onji M. Mechanism and therapeutic potential of DNA-based immunization against the envelope proteins of hepatitis B virus in normal and transgenic mice. *Immunology* 2001; **103**: 90-97
38 **Tacket CO**, Roy MJ, Widera G, Swain WF, Broome S, Edelman R. Phase 1 safety and immune response studies of a DNA vaccine encoding hepatitis B surface antigen delivered by a gene delivery device. *Vaccine* 1999; **17**: 2826-2829
39 **Roy MJ**, Wu MS, Barr LJ, Fuller JT, Tussey LG, Speller S, Culp J, Burkholder JK, Swain WF, Dixon RM, Widera G, Vessey R, King A, Ogg G, Gallimore A, Haynes JR, Heydenburg Fuller D. Induction of antigen-specific CD8+ T cells, T helper cells, and protective levels of antibody in humans by particle-mediated administration of a hepatitis B virus DNA vaccine. *Vaccine* 2001; **19**: 764-778
40 **Weber R**, Bossart W, Cone R, Luethy R, Moelling K. Phase I clinical trial with HIV-1 gp160 plasmid vaccine in HIV-1-infected asymptomatic subjects. *Eur J Clin Microbiol Infect Dis* 2001; **20**: 800-803
41 **Swain WE**, Heydenburg Fuller D, Wu MS, Barr LJ, Fuller JT, Culp J, Burkholder J, Dixon RM, Widera G, Vessey R, Roy MJ. Tolerability and immune responses in humans to a PowderJect DNA vaccine for hepatitis B. *Dev Biol (Basel)* 2000; **104**: 115-119

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