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Expression of hepatitis B virus surface antigen determinants in *Lactococcus lactis* for oral vaccination

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Summary

Lactococcus lactis with non-pathogenic and non-colonizing properties is an attractive candidate for delivering biologically active proteins by mucosal routes. In this report we described recombinant *L. lactis* applicable for the development of live mucosal vaccine against hepatitis B virus (HBV). The *PreS* region of the HBV surface antigen alone or combined with ''a'' determinant of S region (*PreSa*) was cloned and expressed in the food grade bacterium *L. lactis* using a nisin-controlled expression (NICE) system. Western blot analysis indicated that both PreS and PreSa fusion proteins were successfully expressed in *L. lactis* after nisin induction. Oral immunization of BALB/c mice with PreS and PreSa-producing strains induced both mucosal (intestinal IgA) and systemic (serum IgG) immune responses against HBV at the same magnitude. Two additional groups of mice given *L. lactis* expressing human interferon-alpha 2b as an adjuvant with the PreS or PreSa-producing strains produced higher IgG but not IgA antibody responses. These results indicated that the lactococci-derived vaccines could be promising candidates as alternative HBV vaccines for preventing hepatitis B.

Introduction

Hepatitis B virus (HBV) infection is a serious threat to human health worldwide, and causes significant morbidity and mortality in chronic carriers of HBV. Despite the availability of a commercially used yeast-derived HBV vaccine,

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around 5–10% of the vaccinees do not develop protective immunity against the virus (Valenzuela et al., 1982). Additionally, the necessity of intravenous administration and high cost limit its use for large populations in developing countries. Therefore, an effective, inexpensive and easily administered vaccine needs to be developed.

By using *Lactococcus lactis* as an antigen delivery vehicle, the problems described above could be potentially circumvented. *L. lactis* is a gram-positive bacterium with "GRAS" (generally regarded as safe) status, which has been used in fermentation and preservation of food for decades (Nouaille et al., 2003). These food-grade bacteria do not produce lipopolysaccharides or other toxins, and their recombinant products do not require purification. They are also attractive candidates for delivering bioactive proteins through mucosal routes because of their non-pathogenic and non-colonizing properties as well as their abilities to induce immune responses effectively (Wells et al., 1996; Robinson et al., 1997; Pei et al., 2005; Hou et al., 2007; Scavone et al., 2007). To date, a number of antigens of bacterial or viral origin have been produced by *L. lactis* in a form that can be presented to and processed by the immune system of the mammalian host (Le Loir et al., 2005). When the recombinant strains were administered to mice, immune responses could be elicited against these antigens (Xin et al., 2003; Bermudez-Humaran et al., 2004). In the case of mice immunized with *L. lactis* expressing tetanus toxin (TTFC), these responses proved to be protective against lethal challenge with TTFC (Wells et al., 1993; Norton et al., 1997).

The hepatitis B virus surface antigen (HBsAg) is localized to the envelope of HBV, which contains three antigenic domains designated as S, PreS2 and PreS1 (Heermann et al., 1984). The S antigen of HBsAg has been studied in detail and used as a vaccine antigen to prevent infection. The "a" determinant in the S region is common to all four major serological types (adr, adw, ayr and ayw) (Tiollais et al., 1985) of HBV, and can induce neutralizing antibodies against HBV (Waters et al., 1991). When the conformation of the "a" determinant is destroyed by reduction or alkylation, HBsAg loses antigenicity (Mishiro et al., 1980). The PreS1 and PreS2 regions together are known as the PreS domain. Anti-serum against synthetic PreS1 peptide (amino acid 21–47) or PreS2 was able to neutralize the virus and could protect chimpanzees from HBV infection (Itoh et al., 1986; Neurath et al., 1989). In addition, it has been shown that inclusion of the PreS region in a vaccine containing the S region can augmented anti-HBs responses in mice (Shouval et al., 1994).

Many mucosal adjuvants like cholera toxin (CT) or cytokines are required to be co-delivered with vaccines in order to enhance the immune responses (Singh and O'Hagan, 1999). Interferon-alpha (IFN-α) is a cytokine with antiviral, antitumor and immunomodulatory properties. IFN-α has been shown to promote Th1 cell differentiation (Bracci et al., 2008) and is a powerful mucosal adjuvant when administered to mice with human influenza vaccine (Bracci et al., 2005).

In this report, the PreS region of HBsAg as well as the fusion protein including PreS and "a" determinant (PreSa) were expressed in the food grade bacterium *L. lactis* and tested as a candidate vaccine against HBV. At the same time, recombinant *L. lactis* producing human interferon alpha 2b (IFN) was tested as an adjuvant for this vaccine. Our results indicated that serum IgG and intestine IgA were effectively induced in mice orally administered with these recombinant strains.

Materials and methods

Bacterial strains, plasmids and culture conditions

The bacterial strains and plasmids used in this study are listed in Table 1. *Escherichia coli* strains were grown in Luria–Bertani (LB) medium with aeration at 37 °C, while *L. lactis* strains were cultivated in GM17 at 30 °C without shaking. When necessary, antibiotics and inducers were added as follows: for *E. coli*, ampicillin (100 μg/ml), chloramphenicol (10 μg/ml) and isopropyl thiogalactose (IPTG; 1 mM); and for *L. lactis*, Chloramphenicol (5 μg/ml) and nisin (10 ng/ml).

Expression and purification of PreS protein in *E. coli*

For expression and purification of the PreS protein in *E. coli*, the plasmid pGEX-pres was constructed as follows. A 521 bp DNA fragment (nucleotides 1–521), encoding the PreS1 and PreS2 regions, was amplified from plasmid pCMV-LS (containing the full-length HBsAg type adr coding sequence, kindly provided by Professor Yumei Wen, Fudan University) and digested with *Bam*HI and *Xho*I. The purified fragment was then ligated to the same digested pGEX-6p-1 to obtain expression vector pGEX-pres (Figure 1A).

After transformation of *E. coli* BL21 (DE3) with the plasmid pGEX-pres, the recombinant strain was cultured until the mid-exponential phase