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A novel approach of prophylaxis to HBV recurrence after liver transplantation

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ABSTRACT

Liver transplantation (LT) in patients with hepatitis B virus (HBV) infection is associated with a high rate of graft loss and poor survival, unless re-infection can be prevented. Human hepatitis B immune globulin (HBIG) and nucleoside analogues (NA) have long been utilized to prevent re-infection. Previously, we generated a human monoclonal antibody (mAb), HB that recognizes the surface antigen of hepatitis B virus (HBV). We have constructed a secreted version of HB and cloned its genes into recombinant adeno-associated virus (AAV). We compared the efficiency of AAV vector after a single injection via intramuscular or intravenous routes without additional intervention. Then, we evaluated the activity of antibody HB in tree shrews treated with rAAV-HB and in vitro experiments. So, intramuscular injection of rAAV-HB was a suitable method for the immunoprophylaxis of HBV infection. This human antibody will be useful for the immunoprophylaxis of HBV infection.

Chronic hepatitis B virus (HBV) infection occurs worldwide, and more than 10% of the population in hyper endemic areas such as Southeast Asia, China, and Africa are HBV carriers (Andre, 2000; Kane, 1996). The patients infected with Hepatitis B virus (HBV) may incur chronic hepatitis, cirrhosis, and hepatocellular carcinoma (Blum et al., 1998). Liver transplantation (LT) has become a successful surgical therapy for those terminal liver diseases. However, recurrence of HBV infection after LT plays a key role for the outcome after liver transplantation concerning patient and graft survival rates (Freshwater et al., 2008).

It was reported that HBV recurrence after LT is significantly reduced by prophylaxis with hyper immune antibody to hepatitis B surface antigen (anti-HBs) globulins (HBIG) and antiviral drugs (Muller et al., 1991;Tchervenkov et al., 1997;Schiff et al., 2007; Anderson et al., 2007; Ołdakowska et al., 2007). Low-dose intramuscular HBIG protocols have yielded cost reduction by >50%. However, HBIG is still expensive and requires close monitoring of the LT patient. HBIG is prepared from the sera of antibody positive donors and its safety is assured by strict product standards (Habib and Shaikh, 2007; Lo et al., 2003; Brown and Moonka, 2000; Shouval and Samuel, 2000; Muller et al., 1991). Therefore, the currently available HBIG is not an ideal source of therapeutic antibody due to its limited availability, low specific activity and possible contamination of infectious agents (Ehrlich et al., 1992; Witherell, 2002). HBIG-free therapeutic regimens with new promising nucleos (t) ide analogue combinations are currently being investigated for their efficacy and safety as first-line therapy in clinical studies (Wong et al., 2007). Therefore, adoptive gene transfers of immunity against HBV, HBV vaccines and donor-derived immunity need to be elucidated (Coffin and Terrault, 2007; Beckebaum et al., 2008).

The genetically engineered monoclonal antibodies (mAbs) specific to the surface antigens of HBV would be a good alternative for the immunoprophylaxis of HBV infection (Hong et al., 2004) and may have the potential to resolve these problems. Therefore, Fab fragments are particularly important for a wide variety of applications, including detection and treatment of human diseases (Levy and Miller, 1983), in vitro diagnostic test (Tam and Goldstein, 1985) and affinity purification methods (Johnstone and Thorpe, 1982). Because of the absence of the Fc fragment, they are low-molecular-weight molecules with a high degree of specificity, so they easily localize to the target and are rapidly cleared without accumulation in the tissues (Protzer-Knolle et al., 1998; Trautwein, 2004). So, anti-HBsAg Fab fragment has a considerable potential in the prevention and treatment of liver diseases caused by HBV.

Therefore, we cloned the gene of a high affinity antibody, which came from human peripheral blood. In the previous report, human Fabs against HBsAg were selected from phage-displayed antibody library which was constructed using peripheral blood lymphocytes from vaccinated volunteers. The Fab antibody (named

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as HB) gene was inserted to prokaryotic expression vector p3HB. The affinity of HB by competition ELISA was 0.5×10^9 M^{-1} (Yan et al., 2000). The antibody showed neutralizing activity against the Ad subtypes of the virus in an in vitro infection of adult human hepatocyte primary culture by HBV (Tao et al., 2006; Tao et al., 2007). In this study, we have constructed the rAAV-HB, investigated and selected the vector delivery methods, and evaluated its activity. Whereas the gene therapy with rAAV-HB by muscular injection is considered as an effective treatment for immunoprophylaxis of HBV infection, the human antibody HB will be potentially useful.

Results and discussion

Establishment of AAV producing human anti-HBsAg antibody HB

To construct the eukaryotic secreting HB, the cDNA encoding the light chain and the heavy chain of HB, were cloned from pH3B and

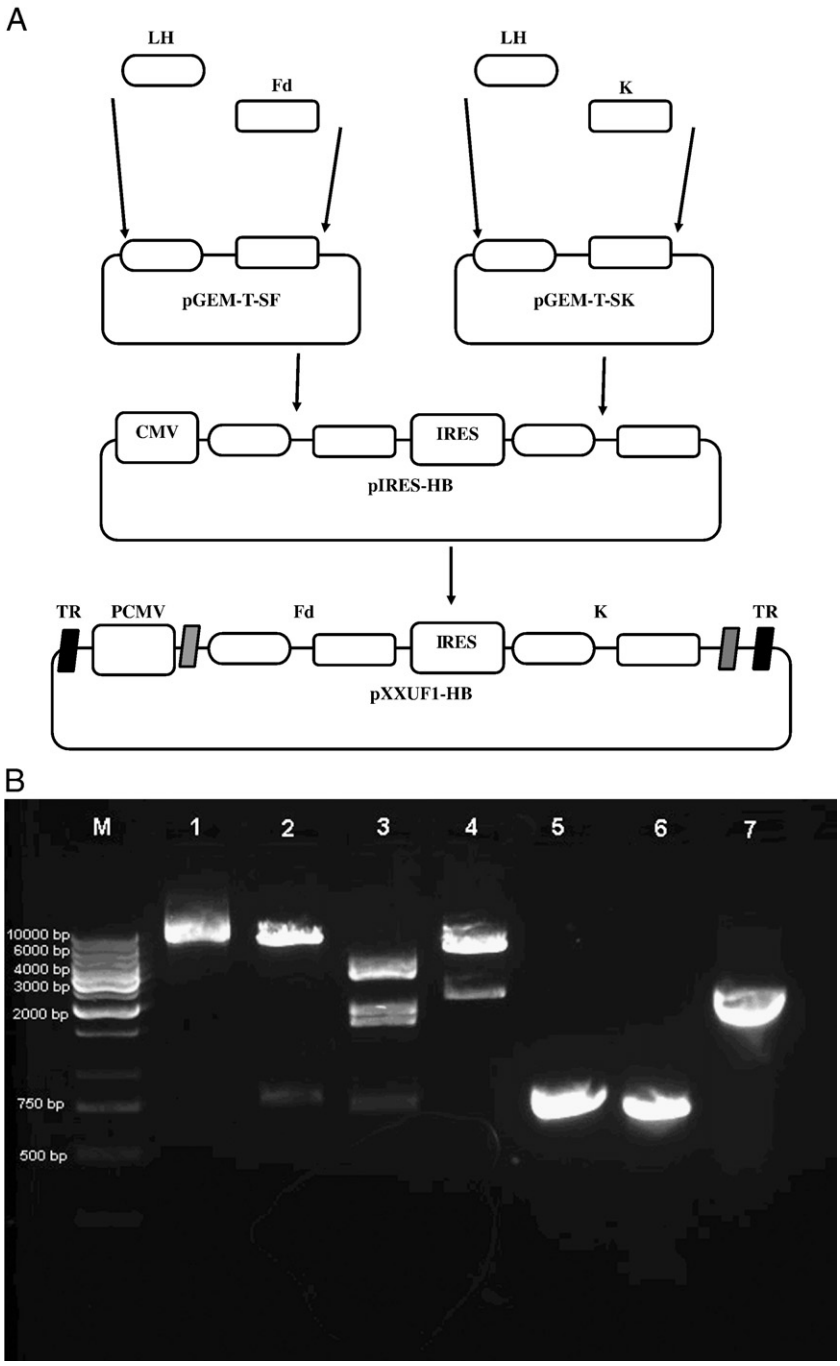

Fig. 1. (A) Schematic representation of the strategy for building pXXUF1-HB. The heavy chain and light chain of Fab were fused with LH by overlap PCR. The resulting fusion fragments were cloned in the vector pGEM-T. The fusion Fd fragment and the fusion K fragment were connected by pIRES after one ribosome-binding site for stable expression of both the fragments. The genes of HB were cloned into pXXUF1. (B) The pXXUF1-HB is identified by enzyme cutting and PCR. M. 1 kb DNA ladder";; 1. pXXUF1-HBs-Fab";; 2. pXXUF1-HBs-Fab/Not I+ Mul I; 3. pXXUF1-HBs-Fab/Xba I+Sal I; 4. pXXUF1-HBs-Fab/Not I+Sal I; 5. PCR: pXXUF1-HBs-Fab/5F+3F; 6. PCR: pXXUF1-HBs-Fab/5K+3K; 7. PCR: pXXUF1-HBs-Fab/5F+3K. It shows that the target genes are successfully cloned into pXXUF1. (C) Nucleotide sequences of the heavy chain (c1) and light chain (c2) of HB antibody. The added leader sequences are underlined. Nucleotide sequences and derived amino acid sequence of the human anti-HBs-Fab V_H and V_L are dashed. The sequence in the AAV remains same with the one in p3HB.