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Generation and Characterization of Human Hepatitis B Virus Specific Antibodies Using Phage Display System and Studies of Their Variable Region Gene Usage

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Hepatitis B virus (HBV) Phage display Specific antibody Hepatitis B surface antigen (HBsAg)

B	(HBV)					HBV	Surface	(HBsAg)	HBV										
B	HBsAg								HBsAg										
				HBsAg						Kappa		Lambda						4.65	10/sup
4/	2.5	10/sup 5/		4	HBsAg					Kappa			46.6%	93.3%					Lambda
				71.4%	100%				50kD				Fab						ELISA
			4		HBsAg	DNA		4	2		2			VH4					
					HBsAg		DNA			HBV			HBV						B
					HBsAg														

Hepatitis B virus (HBV) is one of the major pathogens of chronic hepatitis, which may lead to liver cirrhosis and hepatoma. HBV surface antigen (HBsAg) plays an important role in HBV infection. To further study HBsAg and the possible applications of monoclonal antibodies against the virus, phage display system offers an alternative method for the generation of large numbers of anti-HBsAg mono-specific antibody molecules. In the present study, two phage display antibody libraries were established by combining PCR products of heavy chain genes with those of either kappa or lambda light chain genes, resulting in 4.65 $\times 10^4$ and 2.5 $\times 10^5$ clones in size, respectively. After 4th panning against the purified HBsAg, the results of restriction analysis on randomly selected clones showed that both heavy and light chain gene inserts increased from 46.6% to 93.3% (kappa library) and 71.4% to 100% (lambda library), respectively. Furthermore, the detection of a 50 kD protein molecule using western blotting technique suggested the heavy and light chain polypeptides have been expressed and associated into the correct configuration. The preliminary ELISA data suggested that 4

clones may be specific for HBsAg, but not for BSA. DNA sequences of 4 clones indicated that 2 clones are identical but different from the other 2 identical clones. Whether or not these Fab fragments are specific for HBsAg molecule remains to be determined. Taken together, our data suggested phage display system could offer an efficient way in the generation of virus-specific clones.