論　文　内　容　の　要　旨
Synopsis of Thesis

氏　名 Name	張　赫
論文題名 Title	Establishment of monoclonal antibodies broadly neutralize infection of hepatitis B virus （B型肝炎ウイルスの感染を広範に中和するモノクローナル抗体の作製）

論文内容の要旨

〔目　的(Purpose)〕

The current hepatitis B vaccines consists of recombinant S protein are highly effective, suggesting that antibodies against S protein are able to block HBV infection. Besides, hepatitis B immunoglobulin (HBIG) containing antibodies against S protein are clinically used for HBV protection. However, the use of HBIG is undermined by its high cost and limited availability, and immune pressure mediated by HBV vaccine or HBIG has led to the emergence of immune escape mutants of HBV. We aim to develop a neutralizing antibody that is not only mass-producible, but also prevents HBV infections including escape mutants.

〔方法ならびに成績(Methods/Results)〕

Monoclonal antibodies (mAbs) against S protein were generated by immunization of mice with recombinant S protein. The antigen specificities of mAbs against each HBV genotypes (genotypes A, B, C and D) were confirmed by immunofluorescent staining. However, none of the mAbs recognized S protein by Western blotting, indicating that all these antibodies recognized conformational epitopes but not linear epitopes. To determine the neutralizing activity of the mAbs, HBV of genotype D and the antibodies at 20 μg/mL were simultaneously inoculated into HepG2-NTCP-C4 cells. Intracellular HBV RNA was quantified by qRT-PCR after 10 days and 5 antibodies (mAbs 351, 1531, 1170, 1292, and 1215) were found to suppress HBV infection efficiently. We also examined the neutralizing activity of these mAbs on various HBV genotypes by using HDV, a satellite virus of HBV uses HBV-encoded envelope proteins for entry into hepatocytes. HDVs possessing HBV envelope protein of genotypes A, B, C, and D were used to infect HepG2-hNTCP-18C cells in the presence of the mAbs, and intracellular HDV RNA was determined at 10 days-post infection. All of the 5 mAbs inhibited HDV infection derived from all genotypes of HBV examined. Neutralization activity of each mAbs was determined, and mAbs 351, 1531 and 1170 showed potent neutralizing activities with IC50 around 4-20 ng/ml. Binding activity between mAbs and S protein was measured by BLI and the KD value of these antibodies are in the low nanomolar to picomolar range. Efficacy of mAbs 351 and 1170 against HBV in vivo was confirmed by hydrodynamic injection model. Next, to identify the amino acid residues critical for neutralizing antibodies, we generated several truncation mutants and performed alanine scanning of S protein and revealed that detection of S protein was disrupted by the mutation of Ile152 to Ala, even though this residue is localized outside the "a" determinant, which is thought to be the primary neutralizing antibody binding site. Based on HBV sequences from patients, approximately 99.9% of HBV were shown to possess Ile152 residue in S protein, indicating that Ile152 in S protein is highly conserved, and we found that a mutation in this residue may reduce large hepatitis B surface protein expression. In addition, mAbs 351 and 1531 were found to be able to neutralize the infection of hepatitis D virus possessing Gly145 mutation to Arg in S protein, which is a well-known escape mutation against HBIG treatment. Finally, based on the mouse antibody, we established humanized mAb 351 and demonstrated that it possessing similar affinities and neutralizing activities as the original mouse antibody.

〔総　括(Conclusion)〕

In this study, we generated and characterized several mouse mAbs that target S protein. IIe152 of S protein, which is a highly conserved amino acid, plays crucial role on the interaction with mAbs. Since mAb 351 could successfully neutralize the most well-known G145R escape mutant, we established a humanized antibody mAb 351 which maintains high neutralizing ability and affinity. Consequently, studies to reduce of immunogenicity and enhance neutralization activity of the humanized 351 antibody are worth to try for future clinical applications.

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論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

本論文では、B型肝炎ウイルス(HBV)の主要な抗原であるHBsAgに対するモノクローナル抗体の中から、強い中和活性を有する抗体を選別しその機能解析の結果、HBsAgの認識に152番目のイソロイシンを認識する抗体を同定した。152番目のイソロイシンはHBVの感染だけでなく、HBVのエンベロープ蛋白質の発現、３次元構造に重要な役割を果たしていることを明らかにした。また、そのような抗体の中には、HBIGに対してエスケープ変異として知られているHBsAgの変異に対しても対応できることを発見した。さらに、HBsAgの152番目のイソロイシンを認識する抗体の中から、中和活性能の高かったクローンに関して可変領域を検討し、抗体遺伝子のクローニングとヒト化抗体のデザインを行い、マウス抗体と同等の親和性と中和活性能を有したヒト化抗体の樹立に成功した。このような研究成果は、HBVの肝移植後感染、再活性化予防、母子感染の阻止のために利用されているヒト免疫グロブリン(HBIG)を代替できる中和抗体の開発に資する研究であり、博士（医学）の学位授与に値する。