Claudin-1-specific monoclonal antibodies and their inhibition of hepatitis C virus infection

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ABSTRACT SUMMARY

Claudin-1 (CL-1) is a potent target for drug delivery, cancer treatment, and the prevention of infection by hepatitis C virus (HCV). However, CL-1 binders, such as antibodies (Abs) and chemicals, have never been fully developed. In this study, we tried to develop anti-CL-1 Abs. Mice were immunized with CL-1-expressing cells, budded baculovirus displaying CL-1, or DNA coding CL-1. DNA-immunized mice showed the highest serum titer for anti-CL-1 Abs. Then we prepared hybridoma by using B cells from the DNA-immunized mice, and established four hybridoma clones producing CL-1 Abs. All of the anti-CL-1 Abs specifically bound to CL-1 and prevented infection of human hepatoma cells with HCV. These findings indicate that the CL-1 Abs will be potential seeds for HCV therapy.

RESULTS AND DISCUSSION

However, the low immunogenicity and hydrophobicity of CL-1 make it difficult to generate CL-1 binders, including Abs. In this study, we optimized protocols for immunization and tried to create anti-CL-1 Abs.

EXPERIMENTAL METHODS

BXSB mice were used for immunization. Budded baculovirus that displayed human CL-1 (CL-1) on the membrane, CL-1-expressing cells, or CL-1-encoding plasmid DNA was used as immunogen. Serum anti-CL1 titers were evaluated by flow cytometry using CL-1-expressing HT1080 cells.

B cells isolated from the immunized mice were fused with myeloma cells to produce hybridoma cells. The hybridoma cells producing anti-CL-1 Abs were screened by flow cytometry using Huh7 cells (which are sensitive to HCV infection) and Huh7-derived cells (which are insensitive to HCV infection). The Huh7-derived cells had no expression of CL-1. We selected the hybridoma cells, which bound to Huh7 cells but not Huh7-derived cells.

The specificities of the Abs were investigated by using flow cytometry and human CL-1-, CL-2-, CL-4-, or CL-5-expressing HT1080 cells.

The ability of the Abs to inhibit HCV entry was investigated by using HCV-infected Huh7 cells. Briefly, Huh7 cells were pretreated with Abs for 1 h. Then, the cells were infected with HCV for 2 h. After an additional culture of 4 days, HCV core proteins in the conditioned medium and in the cells were measured by ELISA and immunoblot analysis, respectively.

INTRODUCTION

Hepatitis C virus (HCV), a hepatotropic member of the Flaviviridae family, is the leading cause of chronic hepatitis, cirrhosis and hepatocellular carcinoma¹. Approximately 130~200 million people are estimated to be infected with HCV worldwide. Each year, 3~4 million people are newly infected with HCV². Thus, overcoming HCV is a critical issue for the World Health Organization.

Targets for HCV therapy are classified into inhibition of HCV entry into hepatocytes and of HCV replication in hepatocytes. Although a number of studies have been made on development of agents to prevent HCV replication, inhibitors for HCV entry have never been fully developed³.

CL-1 was identified to be a co-receptor for HCV, indicating that CL-1 may be a potential target for prevention of HCV entry in 2007⁴.
budded baculovirus or CL-1-expressing cells. Therefore, we produced hybridomas from B cells isolated from DNA-immunized mice. Using both Huh7 cells and Huh7-derived cells (CL-1-deficient cells) to screen for CL-1-specific Abs, we finally identified four clones (Fig. 1); these clones all have different VH and VL sequences. Human chimeric Abs containing the VH and VL regions also bound to CL-1-expressing cells, indicating that the variable regions are essential for their interaction with CL-1. Moreover, Abs bound to CL-1-expressing cells but not to those expressing CL-2, CL-4, or CL-5 (Fig. 2).

None of the Abs bound mouse CL-1, whose first and second extracellular loop regions show 93.9% and 89.5% homology, respectively, to those of human CL-1. These results indicate that the Abs are highly specific for human CL-1.

Finally, we investigated the effect of the Abs on infection of Huh7 cells with HCV, a popular model for HCV infection. Treatment of cells with each Ab dose-dependently decreased HCV core proteins in the conditioned medium and in the cells. The Abs had no effect on the cellular viability. Thus, the Abs may be leads for HCV therapy.

CONCLUSION

In this study, we found that DNA immunization may be the best procedure for generating anti-CL-1 Abs. We made success on creation of antagonistic Abs for HCV entry, whose inhibitory activities were $10^{-3}$ higher than those reported previously. The screening system using Huh7 cells and its-derived CL-1-deficient cells may be crucial for creating functional Abs.

REFERENCES


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