In vivo inhibition of hepatitis C virus infection by anti-human claudin 1 monoclonal antibodies

M. Iida1, S. Nagase1, M. Yamashita1, Y. Shirasago2,3, M. Fukasawa2, M. Tada4, A. Ishii4, A. Watari1, K. Yagi1, and M. Kondoh1

1Graduate School of Pharmaceutical Sciences, Osaka University, Osaka, 565-0871, Japan; 2Department of Biochemistry and Cell Biology, National Institute of Infectious Disease, Tokyo, 162-8640, Japan; 3Graduate School of Biological Science, Tokyo University of Science, Chiba, 278-8510, Japan; 4Division of Biological Chemistry and Biologicals, National Institute of Health Sciences, Tokyo, 158-8501, Japan

iida-m@phs.osaka-u.ac.jp

ABSTRACT SUMMARY
More than 170 million people worldwide are infected with hepatitis C virus (HCV). Claudin 1 (CLDN1) is a co-receptor necessary for HCV entry into hepatocytes and thus is an attractive potential target for anti-HCV therapy. We previously developed 4 clones of mouse anti-human CLDN1 antibodies (Abs). In this study, we investigated the inhibitory effects of the Abs on HCV infection. All 4 clones attenuated the infection of Huh7 cells by HCV (genotype 2a) and HCV pseudoviral particles (HCVpp, genotypes 1b and 2a). Two of the four clones also prevented HCV (genotype 1b) infection of human-liver chimeric mice. For future clinical application, we prepared human-mouse chimeric anti-CLDN1 Abs. The chimeric Abs prevented HCV and HCVpp infection of Huh7.5.1 cells as effectively as the mouse Abs. Our findings suggest that mouse and human-mouse chimeric anti-CLDN1 Abs prevent HCV infection and may therefore be promising candidate anti-HCV agents.

EXPERIMENTAL METHODS
The ability of the Abs to inhibit HCV entry was investigated using HCV (genotype 2a) and Huh7 cells. Briefly, Huh7 cells were pretreated with Abs for 30 min and then incubated with HCV for 2 h. After additional culturing for 4 days, the presence of HCV core proteins in the conditioned medium and in the cells was assessed by ELISA and immunoblot analysis, respectively. The presence of HCV RNA in the conditioned medium was assessed by qRT-PCR.

The ability of the Abs to inhibit HCV entry was also investigated in vivo using HCV (genotype 1b) and human-liver chimeric mice. Either anti-CLDN1 Ab or control IgG was injected into mice intraperitoneally. After 8 h, HCV was administered intravenously. The presence of HCV RNA in collected blood was measured by qRT-PCR on days 0, 7, 14, 21, 28, 35, and 42. We also determined the level of
human albumin by latex coagulating nephelometry and the levels of AST and ALT by POP and POD/leuco dye method.

The cDNAs encoding heavy- and light-chain variable regions of the anti-CLDN1 Abs were isolated by PCR and cloned into either a pFUSE-CHIg-hG1 or pFUSE2-CLlglg-hk vector for production of human-mouse chimeric IgG1 Ab. The vectors were then transfected into CHO cells via lipofection. Chimeric anti-CLDN1 Abs were purified from the supernatant using a Protein G column.

RESULTS AND DISCUSSION

The levels of HCV core proteins and RNA in conditioned medium and cells were decreased in a dose-dependent manner following treatment with each Ab (Fig 1). Treatment with anti-CLDN1 Abs also decreased the luciferase activity of HCVpp-infected cells (Fig 2). The Abs had no effect on cell viability.

All 4 human-liver chimeric mice injected with the control Ab were HCV-positive. When the mice were treated with 2 of the 4 anti-CLDN1 Ab clones, one clone protected 3 of the animals from HCV infection after 42 days, whereas the other clone protected only 1 of the mice from infection. Injection of mouse anti-CLDN1 Abs did not adversely affect the serum levels of human albumin, ALT, or AST.

For future clinical application, we prepared human-mouse chimeric anti-CLDN1 Abs and investigated their ability to prevent HCV infection. The chimeric anti-CLDN1 Abs prevented HCV and HCVpp infection of Huh7 cells as effectively as the mouse Abs.

CONCLUSION

In this study, we examined the inhibitory effect of 4 previously generated mouse anti-CLDN1 Abs on HCV infection both in vitro and in vivo. Human-mouse chimeric anti-CLDN1 Abs prepared for clinical use had the same inhibitory effect on HCV infection as mouse anti-CLDN1 Abs, suggesting that mouse and mouse-human chimeric anti-CLDN1 Abs would be promising candidate anti-HCV agents.

REFERENCES

3. Evans, MJ. et al., Gastroenterology 2010, 139, 953-964.

ACKNOWLEDGMENTS

This work was supported by a Health, Labor, and Welfare of Japan Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (24390042); the Adaptable and Seamless Technology Transfer Program through Target-driven R&D, Japan Science and Technology Agency; and the Takeda Science Foundation.