Induction of Fas Ligand and Fas Antigen mRNA Expressions in Interferon- γ Transgenic Mouse Liver

Toshihiro Okamoto¹, Tomio Yamakawa¹, Ken-Ichi Yamamura² and Okio Hino³

¹Research Laboratories, Nippon Chemiphar Co., Ltd., Saitama 341–0005, Japan

²Institute of Molecular Embryology and Genetics, Kumamoto University School of Medicine, Kumamoto 862–0976, Japan

³Department of Experimental Pathology, Cancer Institute, Tokyo 170–8455, Japan

Received July 27, 1998 Accepted August 24, 1998

ABSTRACT—The interferon- γ (IFN- γ) transgenic mouse develops chronic hepatitis. In the present study, lymphoid cell infiltration and expression of the Fas system were studied in the transgenic mouse liver. Morphological analysis showed the infiltration of lymphoid cells at the portal area and necro-inflammation in the lobules. Furthermore, the infiltrating lymphoid cells were found to be present in the areas of piecemeal necrosis and the intralobular region. The polymerase chain reaction revealed induction of Fas antigen and Fas ligand mRNA expressions. The chronic hepatitis in this mouse reflects the profiles of human chronic hepatitis.

Keywords: Transgenic mouse, Fas, Liver

Human chronic active hepatitis has specific features such as the infiltration of T lymphocytes at the portal area and necro-inflammation in the lobules (1, 2). Development of a chronic hepatitis model that well reflects the profile of human chronic hepatitis is difficult. The mouse carrying the interferon- γ (IFN- γ) gene linked to the liver-specific promotor serum amyloid P component develops chronic hepatitis (3). CD8 mRNA is a marker of cytotoxic T-cells (4) and the transgenic mouse induces CD8 mRNA in the liver (5), suggesting a possibility of cytotoxic T-cell infiltration. Perforin and the Fas system are cytotoxic mechanisms of cytotoxic T-cells (6). Furthermore, the Fas system is reportedly expressed in the liver of patients with human chronic hepatitis (2, 7).

The objective of the present study was to determine the mode of lymphoid cell infiltration and expression of the Fas system in the liver of the transgenic mouse. We compared the profiles of chronic hepatitis of this mouse with those in human chronic hepatitis.

Transgenic mice [C57BL/6-TgN(IFN- γ)5 Imeg], which carry the mouse IFN- γ gene linked to the serum amyloid P component gene (3), were used at 48 weeks of age (for morphological study, transgenic mouse was used at 38 weeks of age). All animals were housed and treated in accordance with institutional guidelines. Blood sampling was achieved from a tail vein under anesthesia. Plasma transaminase activity, i.e., that of alanine

aminotransferase (ALT), was determined by the standard photometric method using an automatic analyzer (Model 7060; Hitachi, Tokyo). On sacrifice, liver tissue was removed from each animal, and then the liver was immediately frozen with dry ice and stored at -80°C for RNA analysis. For morphological study, the liver was fixed with 10% phosphate-buffered formalin. Paraffin sections (4- μm) were stained with hematoxylin and eosin (H-E) for microscopic examination. RNA isolation (8) and polymerase chain reaction (PCR) analysis were previously described (5). The gene-specific PCR primers for IFN- γ , tumor necrosis factor- α (TNF- α), CD8 and Fas antigen were previously described (5, 9). The PCR primer for the detection of Fas ligand mRNA was purchased from Continental Laboratory, Inc. (San Diego, CA, USA).

The results were analyzed by means of the F test followed by the *t*-test with Welch's correction.

Plasma ALT was measured and found to be significantly elevated in the plasma from IFN- γ transgenic mouse (ALT (U/L): transgenic mouse, 295 ± 93 , $n=4$; normal mouse 21 ± 3 , $n=4$). These results indicate the induction of hepatitis in the transgenic mouse. IFN- γ and TNF- α are important mediators in the development of hepatitis (10, 11), and previously we reported the augmented expression of these cytokine mRNAs in the transgenic mouse liver (5). We also confirmed these cytokine mRNA expressions here (Fig. 1), suggesting a role of

Normal Transgenic

Fig. 1. RT-PCR analysis of IFN- γ , TNF- α and CD8 mRNA expression in the livers of normal and transgenic mice. The left 4 lanes contain normal liver samples and the right 4 contain transgenic mouse liver samples. Messenger RNAs were amplified with IFN- γ , TNF- α , CD8 and GAPDH (glyceraldehyde-3-phosphate dehydrogenase) gene-specific primers for 30 cycles.

these cytokines in the development of hepatitis. Induction of CD8 mRNA suggested the infiltration of cytotoxic T-

cells in the transgenic mouse liver (Fig. 1). Cytotoxic T lymphoid cells have been implicated in the development of human chronic hepatitis (1, 2). In human chronic active hepatitis, lymphoid cell infiltration was found to be present at the advancing edges of areas of piecemeal necrosis at the portal area and necro-inflammation in the lobules (1, 2). In the present study, lymphoid cell infiltration at the portal area and necro-inflammation in the lobules were revealed in the IFN- γ transgenic mouse liver (Fig. 2). Furthermore, the infiltrating lymphoid cells were found to be present in the areas of piecemeal necrosis and the intralobular region (Fig. 2). Our morphological finding revealed that the mode of lymphoid cell infiltration in the IFN- γ transgenic mouse liver was quite similar to that observed in the liver in human chronic active hepatitis. In addition to the specific feature of the areas of the lymphoid cell infiltration, human chronic hepatitis has other characteristic features such as the expression of the Fas system in the liver (2, 7). In the transgenic mouse liver, both Fas antigen and Fas ligand mRNAs were induced (Fig. 3). Although acute stimulation of the Fas system was reported (12), expression of the Fas system in the

Fig. 2. Histology in the liver of a transgenic mouse. Lymphoid cell infiltration at the portal area (arrow) is present. Necro-inflammation (arrowhead) is present in the lobule (H-E, original magnification $\times 241$).

Normal Transgenic

Fig. 3. RT-PCR analysis of Fas antigen and Fas ligand mRNA expression in the livers of normal and transgenic mouse. The left 4 lanes contain normal mouse liver samples and the right 4 contain transgenic mouse liver samples. Messenger RNAs were amplified with Fas antigen and GAPDH gene-specific primers for 30 cycles and with Fas ligand gene-specific primers for 35 cycles.

liver of a chronic hepatitis model has not been demonstrated previously. Here, the liver of this IFN- γ transgenic mouse was shown to express the Fas system. IFN- γ is known to display its potent effects on gene activation (13, 14). Thus, the direct stimulation by IFN- γ , or by effects secondary to the IFN- γ induced inflammation, may stimulate the expression of the Fas system in the transgenic mouse liver.

The present results clearly show that the chronic hepatitis of the transgenic mouse reflects human chronic hepatitis at least in the following two points: 1) infiltration by lymphoid cells at the portal area and necroinflammation in the lobules and 2) expression of Fas antigen and Fas ligand mRNAs in the liver. Thus, our transgenic mouse continues to be a valuable experimental model for the evaluation of drugs for the treatment of chronic hepatitis.

Acknowledgments

We wish to thank Mr. H. Mitani and Ms. T. Takaraha for their technical assistance and Mr. K. Kinoshita for carefully reading the manuscript.

REFERENCES

- Kerr JFR, Cooksley WGE, Searle J, Halliday JW, Halliday WJ, Holder L, Roberts I, Burnett W and Powell LW: The nature of piecemeal necrosis in chronic active hepatitis. *Lancet* **20**, 827–828 (1979)
- Hiramatsu N, Hayashi N, Katayama K, Mochizuki K, Kawanishi Y, Kasahara A, Fusamoto H and Kamada T: Immunohistochemical detection of Fas antigen in liver tissue of patients with chronic hepatitis C. *Hepatology* **19**, 1354–1359 (1994)
- Toyonaga T, Hino O, Sugai S, Wakasugi S, Abe K, Shichiri M and Yamamura K: Chronic active hepatitis in transgenic mouse expressing interferon- γ in the liver. *Proc Natl Acad Sci USA* **91**, 614–618 (1994)
- Nakauchi H, Nolan P, Hsu C, Huang HS, Kavathas P and Herzenberg LA: Molecular cloning of Lyt-2, a membrane glycoprotein marking a subset of mouse T lymphocytes: Molecular homology to its human counterpart, Leu2/T8, and to immunoglobulin variable regions. *Proc Natl Acad Sci USA* **82**, 5126–5130 (1985)
- Okamoto T, Furuya M, Yamakawa T, Yamamura K and Hino O: TNF- α gene expression in the liver of the IFN- γ transgenic mouse with chronic hepatitis. *Biochem Biophys Res Commun* **226**, 762–768 (1996)
- Nagata S and Golstein P: The Fas death factor. *Science* **267**, 1449–1456 (1995)
- Mita E, Hayashi N, Ito S, Takehara T, Hijioka T, Kasahara A, Fusamoto H and Kamada T: Role of fas ligand in apoptosis induced by hepatitis C virus infection. *Biochem Biophys Res Commun* **28**, 468–474 (1994)
- Okamoto T, Mitsunashi M, Fujita I, Sindh RK and Kikkawa Y: Induction of cytochrome P450 1A1 and 1A2 by hyperoxia. *Biochem Biophys Res Commun* **197**, 878–885 (1993)
- Watanabe-Fukunaga R, Brannan CI, Itoh N, Yonehara S, Copeland NG, Jenkins NA and Nagata S: The cDNA structure, expression, and chromosomal assignment of the mouse Fas antigen. *J Immunol* **148**, 1274–1279 (1992)
- Mizuhara H, Uno M, Seki N, Yamashita M, Yamaoka M, Ogawa T, Kaneda K, Fujii T, Senoh H and Fujiwara H: Critical involvement of interferon gamma in the pathogenesis of T-cell activation-associated hepatitis and regulatory mechanisms of interleukin-6 for the manifestation of hepatitis. *Hepatology* **23**, 1608–1615 (1996)
- Gantner F, Leist M, Lohse AW, Germann PG and Tiegs G: Concanavalin A-induced T-cell-mediated hepatic injury in mice. The role of tumor necrosis factor. *Hepatology* **21**, 190–198 (1995)
- Kondo T, Suda T, Fukuyama H, Adachi M and Nagata S: Essential roles of the Fas ligand in the development of hepatitis. *Nature Med* **3**, 409–413 (1997)
- Samanta H, Engel DA, Chao HM, Thakur A, Garcia-Blanco MA and Lengyel P: Interferons as gene activators. *J Biol Chem* **261**, 11849–11858 (1986)
- Marzio PD, Puddu P, Conti L, Belardelli F and Gessani S: Interferon γ upregulates its own gene expression in mouse peritoneal macrophages. *J Exp Med* **179**, 1731–1736 (1994)