Table 2. (Continued)

peak number	Tg/NTg ratio	protein name	molecular mass (Da)	GI number[b]
		glycolytic system		
59	2.96	Fructose-bisphosphate aldolase B	39548	gi|15723268
62	1.21	Fructose-bisphosphate aldolase B	39548	gi|15723268
77	1.39	PREDICTED: similar to GAPDH	35789	gi|51768209
112	1.64	Lactate dehydrogenase 1, A chain	36475	gi|6754524
		metabolism		
21	1.79	TI-225	14167	gi|1167510
22	2.02	TI-225	14167	gi|1167510
32	2.02	Cystatin B	11039	gi|6681071
72	3.23	Carbonic anhydrase 3	29348	gi|31982861
104	1.83*	Acetaldehyde dehydrogenase (ALDH)	54410	gi|9755362
110	1.82	Aldh2 protein	56502	gi|13529509
115	1.90	Malate dehydrogenase (EC 1.1.1.37)	31692	gi|164543
116	2.10	Argininosuccinate lyase	51707	gi|19526986
		signal transduction		
47	1.70	Phosphatidylethanolamine binding protein	20847	gi|9256572
		amino acid synthesis		
74	2.21	Glycine-*N*-acyltransferase	34076	gi|22122359
		other		
5	2.02	ND***		
6	1.99	ND***		
118	1.97	ND***		
19	3.56	γ-actin	40992	gi|809561
23	1.40*	Diazepam binding inhibitor, splice form 1b	15219	gi|67511482
39	2.80	Saposin	61353	gi|249387
45	2.13	Unnamed protein product	58587, 57007, 52653, 49471	gi|12852157, gi|26345440, gi|26349141, gi|26349459,
49	1.42	Peptidylprolyl isomerase A	17960	gi|71051228
103	1.53*	Sorbitol dehydrogenase precursor	40066	gi|1009706
117	3.03	Unnamed protein product	57614	gi|52787

[a] Peak numbers correspond to those in Figure 1. Asterisks indicate significant differences (two-tailed Student's t test, *$P \leq 0.05$, ** $P \leq 0.01$). ***ND, not detected. [b] GI number is simply a series of digits that are assigned consecutively to each sequence record processed by NCBI. The GI system of sequence identifiers runs parallel to the accession.version system, which was implemented by GenBank, EMBL, and DDBJ in February 1999. Therefore, if the protein sequence changes in any way, it will receive a new GI number. (http://www.ncbi.nlm.nih.gov/Sitemap/samplerecord.html#ProteinIDB).

injection loop, and washed using 0.10% TFA in 2.0% acetonitrile at 30 μL/min using the Switchos pump. Peptides were then separated on a nanoflow column (75 μm i.d. × 15 cm, C18 PepMap) at a flow rate of 170 μL/min, employing a gradient from 5.0% to 60% buffer B (0.10% formic acid in 80% acetonitrile) over a period of 35 min (A buffer: 0.10% formic acid in 2.0% acetonitrile). One-second MS/MS scans were performed on each precursor ion. Ions observed with m/z between 350 and 1250 were fragmented with capillary energies from 1300 to 1800 V. The proteins were identified in accord with the previous method.[15,17] There were several candidates with the same score for the unnamed protein products (peak numbers 33 and 45).

Statistical Analysis. Results are expressed as the mean ± SD. The significance of the difference in means was determined by a two-tailed Student's t test.

Results and Discussion

Validation of The FD-LC–MS/MS Method. With the FD-LC–MS/MS method, more than 500 peaks were obtained from an extract of mouse liver tissue derivatized with DAABD-Cl. Typical chromatograms derived from transgenic and non-transgenic mice are depicted in Figure 1. Only the proteins which expression was estimated to fluctuate between transgenic and non-transgenic mice on the same months were identified after isolation, tryptic digestion, and LC–MS/MS identification of arbitrarily selected peak fractions (113 proteins). As a result, 106 proteins differed between transgenic and non-transgenic mice from 6 to 16 months of age, as summarized in Tables 1–3. The total protein amount required for quantification and identification was only 8.0 μg per injection, and identification of even low-abundance proteins was possible with 40 μg of total protein per injection into an HPLC column. In general, proteome analysis of biological samples labeled with CyDye, ICAT, cICAT, or iTRAQ requires from dozens to hundreds of micrograms of protein samples.[3,7–10,11,13,14,28] The accuracy of the method was acquired based on the reproducibility of the peak heights using peaks 53, 83, and 32 as representatives of the high, medium, and low peaks obtained from each individual mouse. The relative standard deviation (RSD, %) for each between-day peak was less than 16 (high peak), 17 (medium peak), and 23% (low peak) ($n = 3$). The reproducibility of the retention time was also calculated using peak 32. The between-day RSD was 0.41% ($n = 3$). As an additional benefit, the simple apparatus, consisting of a pump, a column, and a fluorescence detector, does not require a complex facility for operation. In this study, we attempted a comprehensive profiling analysis of an 11-h operation to evaluate the utility of the method. After the elution time of a subject protein has been determined, it will be possible to reduce the analysis time for an arbitrary analysis of the subject protein by re-optimizing the separation conditions. It would also be possible to reduce the overall analysis time if we could develop a higher-performance column.

Table 3. Altered Proteins between Tg and NTg Mouse Livers for 16 Months[a]

peak number	Tg/NTg ratio	protein name	molecular mass (Da)	GI number[b]
		Down-Regulated		
		respiration		
4	0.41	Hemoglobin, β adult major chain	15738	gi|31982300
67	0.69	Hemoglobin β	15653	gi|229301
53	0.70	α-globin	15076	gi|49902
108	0.70	Quininoid dihydropteridine reductase	25554	gi|21312520
		protein synthesis		
101	0.54	Regucalcin	33385	gi|6677739
		defense		
16	0.69	SOD	15955	gi|201006
65	0.64	Manganese superoxide dismutase	24662	gi|53450
40	0.53	Thioredoxin 1	11668	gi|6755911
63	0.72	Glutathione peroxidase (GSHPx-1) (Cellular glutathione peroxidase)	22268	gi|121666
75	0.49*	Glycine *N*-methyltransferase	32712	gi|15679953
79	0.61	Glutathione S-transferase, mu 1	25953	gi|61402231
83	0.69	BHMT	44992	gi|62533211
95	0.61	Glutathione *S*-transferase, α 3	25344	gi|31981724
97	0.51	Chain B, Glutathione *S*-Transferase Yfyf Cys 47-Carboxymethylated Class Pi, Free Enzyme	23350	gi|2624496
		fatty acid metabolism (containing β-oxidation)		
36	0.69	Fatty acid-binding protein, hepatic (fragment)	10173	gi|90485
82	0.58	Acetyl-Coenzyme A acyltransferase 2 (mitochondrial 3-oxoacyl-Coenzyme A thiolase)	41831	gi|20810027
107	0.70	Acetyl-Coenzyme A acyltransferase 1	43926	gi|18700004
85	0.32*	Hydroxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-Coenzyme A thiolase/enoyl-Coenzyme A hydratase (trifunctional protein), β subunit (HADHB)	51353	gi|13542763
102	0.58	Peroxisomal acyl-CoA oxidase	74608	gi|2253380
		apoptosis		
3	0.69	Eukaryotic translation elongation factor 1 α 1	50140	gi|13278382
8	0.58*	Ribosomal protein S29, isoform 1	6672	gi|22267962
		glycolytic system		
61	0.79	Fructose-bisphosphate aldolase B	39548	gi|15723269
98	0.42	Enolase 1, α non-neuron	47095	gi|12963491
99	0.58	Enolase 1, α non-neuron	47095	gi|12963491
		metabolism		
32	0.46**	Cystatin B	11039	gi|6681071
68	0.80	Carbonic anhydrase 3	29348	gi|31982861
72	0.53	Carbonic anhydrase 3	29348	gi|31982861
109	0.30**	PREDICTED: Carbamoyl-phosphate synthetase 1 (CPS1)	165705	gi|51705066
84	0.50	Argininosuccinate synthetase	46555	gi|6996911
		signal transduction		
87	0.65	Electron transferring flavoprotein, α polypeptide	35018	gi|13097375
		amino acid synthesis		
80	0.39	4-Hydroxyphenylpyruvate dioxygenase	45054	gi|849053
		other		
38	0.54	Histidine triad nucleotide binding protein 1	13768	gi|33468857
49	0.71	Peptidylprolyl isomerase A	17960	gi|71051228
64	0.69	Nit protein 2	30483	gi|12963555
86	0.64	γ-actin	40992	gi|809561
96	0.50	Unknown (protein for IMAGE:6414729)	50209	gi|53734652
103	0.70	Sorbitol dehydrogenase precursor	40066	gi|1009706
106	0.68	Heat-responsive protein	18462	gi|1255116
48	0.59	Unnamed protein product	65586	gi|12859782
		Up-Regulated		
		respiration		
37	1.33	α-globin	15076	gi|49900
		fatty acid metabolism (containing β-oxidation)		
35	1.60	Fatty acid-binding protein, hepatic (fragment)	10173	gi|90485
		other		
17	1.30	ND***		
33	1.34	Unnamed protein product	57807, 58587, 57007, 52653	gi|12852157, gi|26345440, gi|2634914, gi|26349459

[a] Peak numbers correspond to those in Figure 1. Asterisks indicate significant differences (two-tailed Student's *t* test, *$P \leq 0.05$, **$P \leq 0.01$). ***ND, not detected. [b] GI number is simply a series of digits that are assigned consecutively to each sequence record processed by NCBI. The GI system of sequence identifiers runs parallel to the accession.version system, which was implemented by GenBank, EMBL, and DDBJ in February 1999. Therefore, if the protein sequence changes in any way, it will receive a new GI number. (http://www.ncbi.nlm.nih.gov/Sitemap/samplerecord.html#ProteinIDB).

Differential Profiling. Differential profiling analysis was performed using liver tissue from HCV core gene transgenic and non-transgenic mice as model samples to evaluate the feasibility of the FD-LC–MS/MS method for clinical proteomics. To investigate the differential expression of proteins in transgenic and non-transgenic mice, the heights of the peaks corresponding to specific retention times were compared for each month of age, with 106 altered proteins observed. The differentially expressed proteins were classified by age, regulation and function (see Tables 1–3). Tg/NTg ratios over 1.2 were defined as up-regulated, and those below 0.8 were defined as down-regulated. Many proteins were up- or down-regulated during the progression of HCV-associated liver disease. Fifteen proteins were significantly altered in their levels of protein contents (Figure 2). At the age of 6 months, there were fewer down-regulated proteins than up-regulated (9 vs 19 proteins). In contrast, many kinds of proteins were different between transgenic- and non-transgenic mice at 12 months, with 11 proteins being down-regulated and 65 being up-regulated. At 16 months, there were more down-regulated proteins than in any other months (39 proteins), but only a small minority (four) of proteins were up-regulated.

The remarkable decrease in major urinary protein (MUP) and eukaryotic translation elongation factor 1 α 1 (EF-1α1) seen in Figure 2a represents an early event in the progression of HCV-associated liver disease (at 6 months). MUP has been known as a negative tumor marker.[23] Suppression of EF-1α1 expression prevents the induction of apoptosis, with the regulation reflected in an antiapoptotic mode.[24] Although one of the α-globin peaks (peak no. 52) decreased significantly, the other three peaks of α-globin (peak nos. 27, 29, and 37) tended to increase (see Table 1). The expression of α-globin has been shown to be up-regulated in apoptotic stimuli.[25] Therefore, the phenomenon might be considered a trend in apoptosis at this stage. Another observation made at the age of 6 months was the up-regulation of enzymes related to β-oxidation.

At 12 months of age, proteins related to respiration, the electron-transfer system, and defense against reactive oxygen species (ROS) were significantly up-regulated (Figure 2b). Moreover, a majority of proteins involved in respiration, protein synthesis, defense, apoptosis, the glycolytic system, and metabolism were more up-regulated than the changes observed at 6 months (Table 2).

Finally, at 16 months, proteins related to defense, β-oxidation, and apoptosis significantly decreased. Cystatin B[26] and carbamoyl-phosphate synthetase 1 (CPS1)[27] are known to be down-regulated in tumor and/or carcinoma and exhibited a significant decrease with the proposed method (Figure 2c). It was also established that various biological functions such as respiration, protein synthesis, defense, and metabolism tended to decline (Table 3).

As a whole, the investigation of the differential expression of proteins in transgenic and non-transgenic mice revealed that many proteins related to biological functions such as respiration, protein synthesis, defense, β-oxidation, and apoptosis fluctuate during the progression of chronic hepatitis C. These changes may reflect a gross effect derived from the loss of liver function in the various stages of chronic hepatitis in HCV infection.

Additionally, these data support, from the viewpoint of proteomics, the former results obtained from morphological and biochemical observation.[19–21] For example, previous reports suggested that HCV core protein might affect a specific pathway in the lipid metabolism.[19,21] In fact, the core protein has a specific effect on lipid metabolism; fat droplets are formed and accumulate in the liver, leading to steatosis. An analysis of the composition of these lipid droplets determined that the concentration of carbon 18 monosaturated (C18:1) fatty acids, such as oleic and vaccenic acid, significantly increased in the livers of transgenic mice as well as in chronic hepatitis C patients.[21] In the present study, hydroxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-Coenzyme A thiolase/enoyl-Coenzyme A hydratase (trifunctional protein) β subunit (HADHB), which catalyzes fatty-acid metabolism, significantly decreased after 16 months (Figure 2). However, at 12 months, other enzymes associated with β-oxidation tended to increase (Table 2: peak nos. 7 and 82). In addition, up-regulation of ATP synthase led to an increase in the synthesis and metabolism of fatty acid at 12 months (Figure 2). Furthermore, acetaldehyde dehydrogenase (ALDH), which catalyzes the acetaldehyde metabolism, tended to be up-regulated in the same month (Figure 2). The metabolic reactions of fatty acid and acetaldehyde generate $NADH_2^+$, and the overexpression then causes suppression of both metabolisms. Hence, these results suggest that the fatty-acid metabolism may become milder and resulted from the multiple protein changes related to β-oxidation, ATP synthase, and acetaldehyde metabolism with the progression of HCV-associated liver disease.

Previous reports also suggested that HCV core protein might alter the oxidant/antioxidant state in the liver.[20] The reports demonstrated that there is no significant difference in the levels of lipid peroxidation at 3 and 12 months of age, resulting in cellular and tissue damage by ROS. In contrast, after 16 months, the peroxidation and hydrogen peroxide levels increased remarkably and the levels of total and reduced glutathione, which plays an important role as an antioxidant, decreased. While, our results demonstrate that enzymes related to the antioxidant effect, such as betaine-homocysteine methyltransferase (BHMT) and Cu/Zn-superoxide dismutase (SOD), were up-regulated in transgenic mice at 12 months (Table 2: defense). Subsequently, up to 16 months, a decrease in BHMT and glycine *N*-methyltransferase related to the methylation cycle was observed (Figure 2). The decrease in these enzymes led to a deficiency of adenosylmethionine, impairing mitochondrial function and generating oxidative stress in the liver.[29,30] It has recently been shown that a chronic deficiency of adenosylmethionine in the liver results in the spontaneous progression of steatohepatitis and HCC.[31] In addition, the down-regulation of glycine *N*-methyltransferase would inhibit the synthesis of glutathione resulting in a shift to the oxidizing state, thereby reducing cell proliferation and increasing apoptosis.[32] Therefore, the observed expression of antioxidants might reflect direct oxidative stress status; although at 12 months the up-regulated antioxidants protected against oxidative stress, the oxidative stress might become dominant by the deficiency of antioxidants among the progression of liver disease. Also, these results, derived from both studies, strongly suggest that HCV core protein induces ROS in an age-dependent manner. After 16 months, a biochemical[20] and proteomic analysis revealed a lack of glutathione, suggesting that supplying glutathione might be more effective than SOD in the progression of HCC in the late stage. Although a further animal experiment should be required for reliable clarification of the hepatocarcinogenesis mechanism, the proposed method was demonstrated to be extremely

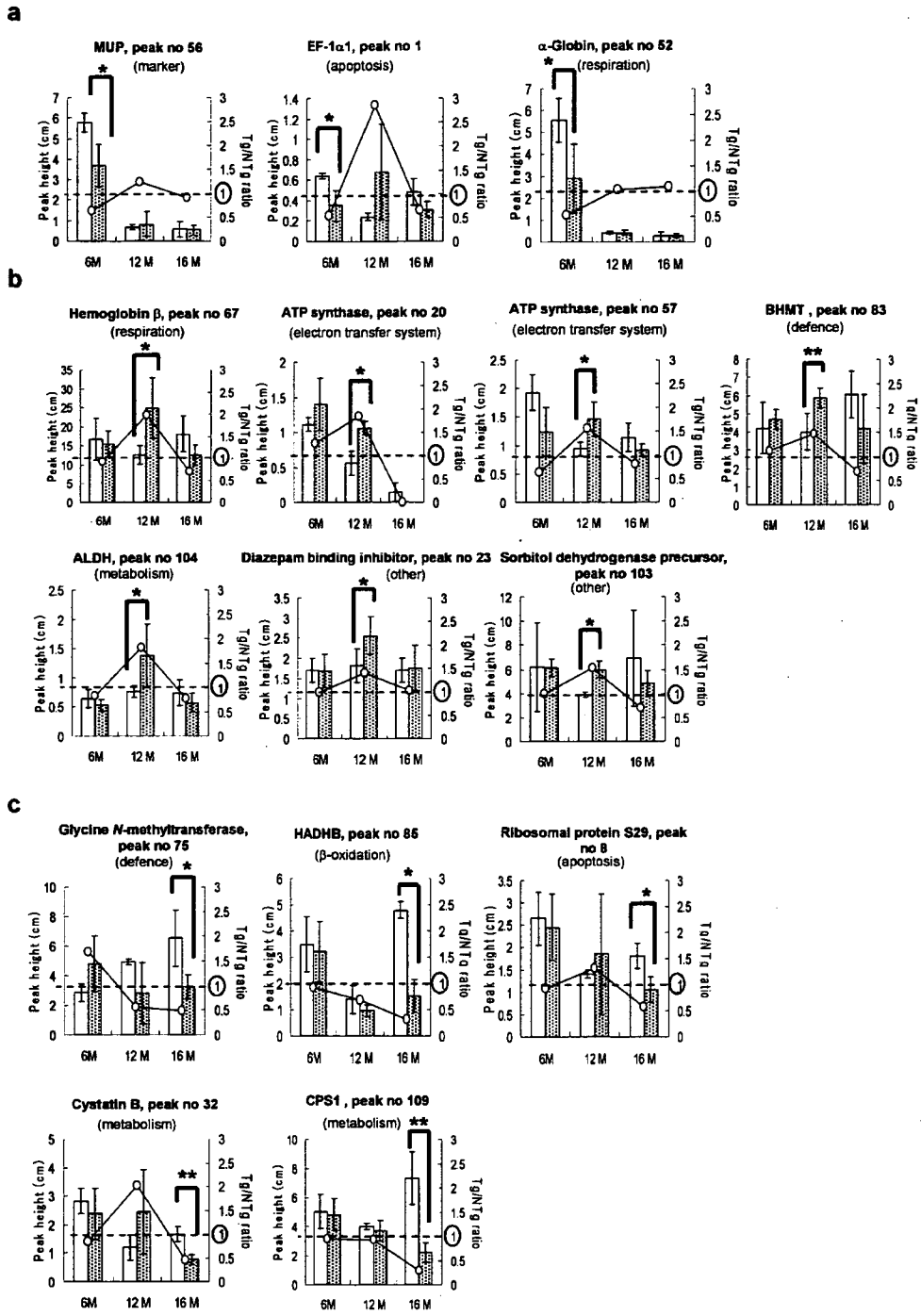

Figure 2. Comparison of peak heights between transgenic (Tg; gray bar) and non-transgenic (NTg; white bar) mice, and the Tg-to-NTg ratio (open circle) from 6 months (6M) to 16 months (16M). Significantly altered proteins are seen at 6M (a), 12M (b), and 16M (c). Peak numbers correspond to those in Figure 1. Mean values ± SD are plotted. Asterisks indicate significant differences (two-tailed Student's *t* test of all data points, $*P \leq 0.05$, $**P \leq 0.01$).

useful for understanding biotransformation from the viewpoint of proteomics. Also, the data obtained in this experiment could support the understanding of hepatocarcinogenesis with HCV infection in terms of proteomics in addition to the morphological and biochemical observations mentioned above.

Conclusions

The proposed method demonstrated for the first time the existence of several event-marker proteins at the three progression stages of hepatocarcinogenesis in transgenic mice. It should be stressed that the FD-LC−MS/MS method would also

be worthwhile for clinical proteomics analysis, as a supplement to gel- and LC-based methods.

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Review

Hepatitis C as a systemic disease: virus and host immunologic responses underlie hepatic and extrahepatic manifestations

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Key words: extrahepatic legion, HCV, immunoglobulin

Introduction

Hepatitis C virus (HCV) causes liver diseases. Approximately 2 million people in Japan and approximately 170 million people worldwide are infected with HCV, and they often suffer from chronic hepatitis, followed by hepatic cirrhosis, leading to hepatic cancer. It was determined relatively soon after the discovery of HCV that HCV infection does not involve the liver only. Other than hepatitis, many complicating diseases of the organs and tissues other than the liver, referred to as extrahepatic lesions, occur in association with HCV infection (Table 1). This review provides an overview of typical extrahepatic lesions associated with hepatitis C.

Cryoglobulinemia

Cryoglobulins are abnormal immunoglobulins that solidify into white deposits at 4°C and liquefy at 37°C.[1] The etiology of cryoglobulinemia in HCV infection has not yet been clarified. However, the involvement of apoptosis suppression by B lymphocytes, which produce monoclonal IgM, induced by the association of *bcl-2* and *IgJ(H)* as a result of the translocation of chromosome t(14:18), is suspected. Intrahepatic growth of CD5- and CD81-positive B lymphocytes has been observed, suggesting monoclonal IgM induction as a possible cause.[7]

Cryoglobulins are classified into three types, namely, monoclonal cryoglobulins (type I), polyclonal cryoglobulins (type III), and mixed cryoglobulins (type II). Cryoglobulinemia associated with HCV infection mainly involves the mixed type. More specifically, it involves monoclonal IgM and polyclonal IgG antibodies having rheumatoid factor activity.[8,9]

The clinical symptoms of essential mixed cryoglobulinemia (EMC) include purpura, arthralgia, and renal impairments.[10] Renal impairments are particularly known for showing membranoproliferative glomerulonephritis histologically and progressing to renal insufficiency.[11] Approximately 80% of EMC patients are infected with HCV.[12] When the high-sensitivity gel diffusion method is used, cryoglobulins are detected in 70% of patients chronically infected with HCV.[13] Many patients with HCV-associated cryoglobulinemia show subclinical symptoms, but the incidence of EMC is highest as an extrahepatic complication of hepatitis C.

Interferon (IFN) therapy has been used for HCV-associated cryoglobulinemia.[14] Misiani et al.[15] reported that, following the administration of IFN to 25 patients with HCV-associated cryoglobulinemia, cryoglobulinemia symptoms improved in 15 patients after the start of treatment but that the symptoms recurred after treatment ended. The combination of IFN and ribavirin has become standard therapy for chronic hepatitis C. It has also been used to treat HCV-associated cryoglobulinemia, with particular efficacy expected in patients for whom IFN monotherapy is ineffective. Zuckerman et al.[16] reported that the administration of both IFN and ribavirin to nine EMC patients who had not responded to IFN monotherapy alleviated cryoglobulinemia in all and improved clinical symptoms in seven of the nine patients.

In addition, for patients with severe cryoglobulinemia, antiviral therapy based on IFN and combination therapy with a steroid or an immunosuppressant are considered effective.[17] Other treatment strategies, including plasma exchange therapy[17] and splenectomy,[18]

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Table 1. Extrahepatic manifestations of chronic hepatitis C

Complication	Pathogenesis	Prevalence of HCV antibody (%)	Treatment with antiviral drug	References
Cryoglobulinemia	Apoptosis suppression of B lymphocytes. monoclonal IgM production caused by translocation of chromosome t(14:18)	50–90	Interferon Pegylated Interferon plus ribavirin	1–17
Renal impairment	Accumulation of an immune complex formed by monoclonal or polyclonal IgM-κ with rheumatoid factor activity produced by HCV-infected B lymphocytes in the glomerular vascular endothelium and mesangium	10–60	Interferon Pegylated Interferon plus ribavirin	18–26
Myocardial impairment	Involvement of host immunologic responses to HCV, particularly human MHC class II antigen	6–10	Not reported	27–31
Porphyria cutanea tarda	Reduced activity of uroporphybrinogen decarboxylase associated with an excessive deposition of iron in the liver induced by HCV infection	60–100	Interferon	32–37
Sjögren's syndrome	Involvement of host immunologic responses to HCV	0–45	Not reported	38–43
Lichen planus	Involvement of HCV-specific T cells	0–65	Interferon	44–63
Oral cancer	Unknown	70–100 (HCV-RNA)	Not reported	64–65
Diabetes mellitus	Involvement of insulin resistance and insulin secretory deficiency. Disruption of tyrosine phosphorylation of IRS-1. Involvement of TNF-α	50	Not reported	66–77
Malignant lymphoma	Involvement of *myc* gene mutation in some cryoglobulinemia patients	0–33	Interferon Pegylated Interferon plus ribavirin	78–94
Autoimmune thyroid disease	Involvement of LKM1	10	Not reported	95–102
Idiopathic interstitial pneumonitis	Involvement of activated T lymphocytes and eosinophils	28	Not reported	103–107
Mooren's ulcer	Unknown	Unknown	Not reported	108–114

HCV, hepatitis C virus; MHC, major histocompatibility; IRS, insulin receptor substrate; TNF, tumor necrosis factor; LKM1, liver/kidney microsomal antibody 1

have also been attempted, and future development of these strategies is promising.

Renal impairments

Reported renal impairments associated with HCV infection include membranoproliferative glomerulonephritis, membranous nephropathy, mesangial proliferative glomerulonephritis, Henoch-Schönlein purpura nephritis, and tubulointerstitial nephritis.[19]

Membranoproliferative glomerulonephritis, in particular, is considered a typical example of hepatic disease involving renal impairment associated with HCV and is referred to as HCV-associated nephritis. In 1993, Johnson et al.[11] first reported on eight patients with HCV infection complicated by membranoproliferative glomerulonephritis.[11] The incidence of HCV-associated nephritis developing as a complication of hepatitis C has not been confirmed. In a study of 188 autopsied cases of chronic hepatitis C, Arase et al.[20] reported that 11.2% of patients exhibited membranoproliferative glomerulonephritis, 2.7% membranous nephropathy, and 17.6% mesangial proliferative glomerulonephritis. The pathogenic mechanism underlying HCV-associated nephritis is considered to be the accumulation of an immunocomplex formed by monoclonal or polyclonal IgM-κ with rheumatoid factor activity produced by HCV-infected

peripheral blood B lymphocytes in the glomerular vascular endothelium and mesangium.[21]

Histopathological features of HCV-associated nephritis are similar to those of typical membranoproliferative glomerulonephritis type I, but the former sometimes show cryoglobulin deposition.[22] In essential cryoglobulinemia and nephrotic syndrome with a rheumatoid factor, HCV-associated nephritis is suspected; therefore, the presence or absence of HCV infection should be determined.

IFN therapy has been reported to be efficacious for HCV-associated nephritis.[23,24] Johnson et al.[23] reported that the administration of IFN to 14 patients with HCV-associated nephritis improved proteinuria, but they observed a relapse of nephritis in association with HCV reexpression after the end of IFN therapy in many patients.[23] Recently, IFN and ribavirin combination therapy, which shows a low relapse rate, has been tested.[25,26] Sabry et al.[26] reported on the effectiveness of IFN and ribavirin combination therapy administered to 16 patients with HCV-associated nephritis for whom IFN monotherapy had proved ineffective; a follow-up study is awaited. Steroid and cyclophosphamide have been used for immunosuppression therapy, but satisfactory results using an immunosuppressant alone have not yet been obtained.[27] Because patients with HCV-associated nephritis have been reported to have a poor prognosis,[11] early establishment of a therapeutic procedure based mainly on IFN and ribavirin combination therapy is desirable.

Myocardial impairments

Myocardial impairments for which a causal relationship with HCV infection has been suspected to date include dilated cardiomyopathy, hypertrophic cardiomyopathy, arrhythmogenic right ventricular dysplasia cardiomyopathy, and chronic myocarditis.[28-30]

A study by Matsumori[28] observed positivity for serum anti-HCV antibody in 6.3% (42/663) of patients with hypertrophic cardiomyopathy and in 10.6% (74/697) of patients with dilated cardiomyopathy. These positivity rates were higher than the rate (2.4%) observed among age-matched Japanese blood donors.[28] Positive- and negative-strand HCV RNAs were detected in cardiac muscle samples of these patients, indicating potential intramyocardial HCV multiplication.[29,30] HCV RNA has also been detected in cardiac muscle samples of patients with arrhythmogenic right ventricular dysplasia cardiomyopathy and chronic myocarditis, indicating that HCV potentially plays an important role in the onset of myocardial impairments.[32]

With regard to the cause of myocardial impairments associated with HCV, the involvement of host immunologic responses to HCV, particularly that of the human major histocompatibility (MHC) class II antigen, has been suggested.[30] There are many patients with normal liver enzyme levels among hepatitis C patients with a concomitant myocardial impairment.[28] No established therapy is currently available, but the use of IFN-based antiviral therapy should be considered.

Porphyria cutanea tarda

Porphyria cutanea tarda is an acquired condition in which patients exhibit solar photosensitivity and hepatic damage owing to decreased activity of uroporphyrinogen decarboxylase in the liver.[33] The involvement of alcohol, excess iron, and medications for hepatic impairments in porphyria cutanea tarda was previously considered. However, because HCV infection has been observed in 60%–100% of cases of porphyria cutanea tarda, the involvement of HCV infection in the pathogenesis of porphyria cutanea tarda is suspected.[34]

The mechanism underlying the pathogenesis of porphyria cutanea tarda associated with HCV infection has not yet been clarified. It is assumed, however, that porphyria cutanea tarda results from reduced uroporphyrinogen decarboxylase activity associated with excessive deposition of iron in the liver as a result of HCV infection.[34]

The efficacy of IFN therapy for the treatment of porphyria cutanea tarda has been demonstrated, in addition to avoidance of sun exposure, abstention from alcoholic beverages, and blood letting. Okano et al.[38] reported that IFN therapy given to porphyria cutanea tarda patients with HCV infection led to transaminase normalization, HCV RNA disappearance, and normalization of porphyrin and ferritin levels with improvement of clinical symptoms, including vesicle formation and hypertrichosis. These results demonstrate the efficacy of IFN therapy for porphyria cutanea tarda.

Sjögren's syndrome

Sjögren's syndrome is an aggregate of symptoms characterized by insufficient tear production by the lacrimal glands and insufficient saliva production by the salivary glands because of exocrine lymphocyte infiltration, causing dryness of the eyes and mouth. Patients with Sjögren's syndrome are classified roughly into two groups, those exhibiting only dryness and those exhibiting both dryness and connective tissue disease symptoms such as arthralgia.[39]

An association of Sjögren's syndrome with viral infection has been reported for some time, and 0%–45% of Sjögren's syndrome patients test positive for

anti-HCV antibody.[40] Differences in the anti-HCV antibody positivity rate are attributed to regional differences in the HCV infection rate. Koike et al.[41] verified that transgenic mice with the 1b HCV envelope genotype developed sialadenitis resembling Sjögren's syndrome. Takamatsu et al.[42] detected HCV RNA in salivary gland tissue from anti-HCV antibody-positive patients with Sjögren's syndrome by reverse transcriptase-polymerase chain reaction (RT-PCR) analysis. Arreita et al.[43] performed in situ hybridization of 19 salivary gland tissue samples obtained from eight anti-HCV antibody-positive patients and 11 anti-HCV antibody-negative patients with chronic sialoadenitis or Sjögren's syndrome, and detected HCV RNA in all salivary gland tissue samples from the anti-HCV antibody-positive patients. Moreover, the HCV-infected salivary gland epithelium showed viral multiplication.[43] These reports indicate that HCV plays some role in the development of sialoadenitis in Sjögren's syndrome, but it has not yet been determined whether HCV itself or immunologic responses to HCV infection induce sialoadenitis.

Current therapies for Sjögren's syndrome mainly aim to alleviate the symptoms. Artificial lacrimal fluid and artificial saliva are used to alleviate dryness, and a non-steroidal anti-inflammatory drug or a steroid is administered for treatment of fever and articular symptoms.[39] There are no reports regarding the efficacy of IFN therapy for HCV-associated sialadenitis,[44] and it is necessary to establish a treatment protocol in the future on the basis of accumulated case reports.

Lichen planus

Lichen planus is an inflammatory disease associated with abnormal chronic dermal and intraoral keratinization of unknown etiology. The assumed causes of lichen planus include viral or bacterial infection, immunologic responses, circulatory disorder, allergy, mental stress, abnormal autonomic function, medication, and glucose metabolism disorder.[45,46]

There are many reports of a relationship between lichen planus and HCV infection, but the anti-HCV antibody positivity rate in lichen planus shows marked regional differences, ranging from 0% to 65%.[47–53] HCV reproduction in the skin and oral mucosal epithelium has been examined by in situ hybridization and RT-PCR analysis.[54–56] HCV-specific T cells are reported to be associated with the pathogenesis of lichen planus,[57] but its pathogenesis is not associated with HCV level, genotype, or pathologic severity.[58,59]

The intravenous administration of a glycyrrhizinate preparation has been demonstrated to have efficacy for treatment of HCV-associated lichen planus.[60] Antiviral therapy based on IFN has also been attempted recently and has been reported to be effective,[61] but other investigators have reported that IFN is a lichen-planus-inducing factor[62] or that it can be aggravating factor.[63] No definite conclusion on the effectiveness of IFN against lichen planus is possible. Nagao et al.[64] reported that when intraoral lichen planus lesions in chronic hepatitis C patients administered IFN were observed over time, no macroscopic changes were observed in the lesions 1 year after the end of IFN administration, but that macroscopic and histological improvements were observed 3 or more years after the end of IFN administration. They also assumed that, since positive-strand HCV RNA was detected in the oral mucous membrane of some patients despite the demonstration of histological recovery from lichen planus following IFN therapy, host immunologic responses to HCV infection were related to the development of oral lichen planus.[64] The early establishment of a treatment procedure for lichen planus is desired, because lichen planus is also considered to be a precancerous condition.[45,46]

Oral cancer

A relationship between HCV infection and oral cancer was first reported by Nagao et al.[65] They showed that the HCV infection rate was higher in oral cancer patients than in esophageal, gastric, or colorectal cancer patients.[65] The HCV infection rate has also been found to be higher in patients with cervical squamous cell carcinoma than in controls.[66] When HCV-RNA was examined in cancer tissues from 17 oral cancer patients by RT-PCR analysis, positive-strand HCV RNA was detected in all anti-HCV antibody-positive patients and negative-strand HCV RNA was detected in 71.4% of the anti-HCV antibody-positive patients.[55] These findings interestingly indicate the possibility of HCV multiplication in cancer tissue. No definite conclusion has been arrived at regarding the relationship between oral lichen planus and oral cancer. However, because lichen planus is considered precancerous, as mentioned above, oral examination is also important for patients with chronic hepatitis C.

Diabetes mellitus

In 1994, Allison et al.[67] reported a relationship between HCV-associated cirrhosis and diabetes mellitus, because the rate of diabetes mellitus complication in patients with both cirrhosis and HCV infection was 50%, which is much higher than that (9%) in patients with cirrhosis but without HCV infection. A large-scale epidemiologic survey showed that the rate of non-insulin-dependent

diabetes mellitus occurring as a complication of chronic hepatic diseases associated with HCV infection was higher than that of other chronic hepatic diseases, and that anti-HCV antibody-positive patients aged 40 years or more had a 3.77-fold higher risk of becoming diabetic than anti-HCV antibody-negative patients.[68] In addition, it has been demonstrated that complication by diabetes mellitus is both a risk factor for hepatocellular carcinoma[69] and a prognostic factor in cirrhosis patients.[70] These reports suggest a correlation between HCV infection and type 2 diabetes. Increased insulin resistance and insulin secretory deficiency are considered to be highly involved in the pathogenesis of type 2 diabetes.[71] Petit et al.[72] reported that insulin resistance increases even in chronic hepatitis C patients with slight hepatic impairment and that the index of impairment (HOMA-IR) correlates with the severity of the liver tissue disorder. Tumor necrosis factor (TNF)-α, which closely correlates with hepatic inflammation and fibrillation in chronic hepatitis C,[73] is considered to enhance glucose uptake in peripheral tissue and to promote gluconeogenesis in the liver, leading to the induction of insulin resistance.[74] Shintani et al.[75] confirmed that in transgenic mice with the 1b HCV core genotype, tyrosyl phosphorylation of the insulin receptor substrate 1 in the insulin signal transduction pathway is disrupted and that this disruption causes gluconeogenesis inhibition by insulin in the liver, leading to the induction of marked insulin resistance. These transgenic mice exhibited a high anti-TNF-α antibody level, and insulin resistance was improved by the administration of an anti-TNF-α antibody. These results indicate a close relationship between HCV infection and the pathogenesis of diabetes mellitus. The relationship between HCV infection and hepatocyte fat modification has also attracted attention.[76] Moriya et al.[77] suggested the possible direct involvement of HCV core protein in hepatocyte fat modification, because they observed hepatocyte fat deposits in transgenic mice expressing the HCV core gene. In summary, the above-described findings strongly indicate that hepatitis C has the characteristics of a metabolic disease, and nutritional management is also considered important in the treatment of chronic hepatitis C.

Malignant lymphoma

HCV reproduces in lymphocytes, and studies of a short-term HCV culture system using lymphocytes have been reported.[78,79] Infected lymphocytes may undergo malignant transformation, leading to the development of malignant lymphoma. HCV infection is considered to be associated with the development of malignant lymphoma, particularly in association with the pathogenesis of non-Hodgkin B-cell lymphoma, and many reports suggest a relationship between HCV infection and malignant lymphoma.[80–88] It has been assumed that some cryoglobulinemia patients develop non-Hodgkin B-cell lymphoma in association with *myc* gene mutation.[89] The anti-HCV antibody positivity rates in patients with non-Hodgkin B-cell lymphoma range from 0% to 33%.[80–88] These differences in HCV antibody positivity rates are considered to relate to regional differences in the HCV infection rate. The HCV antibody prevalence tends to be higher in Japan and Italy but lower in Britain and Canada. Studies indicating a relationship between HCV infection and malignant lymphoma have been reported by Ferri et al.[90] and De Vita et al.[91] Ferri et al.[90] reported that 14 of 500 patients with chronic hepatitis C were complicated with non-Hodgkin B-cell lymphoma, and they detected HCV RNA in peripheral blood lymphocytes in all of these patients. De Vita et al.[91] detected positive-strand and negative-strand HCV RNAs in the parotid glands of patients with parotid non-Hodgkin B-cell lymphoma associated with HCV infection, and confirmed the presence of HCV in the parotid gland by in situ hybridization.[91] As shown by these findings, many patients with HCV-associated non-Hodgkin B-cell lymphoma show involvement of extranodal sites such as the liver and salivary glands.[92]

Treatment of HCV-associated malignant lymphoma is similar to that of HCV-associated non-Hodgkin B-cell lymphoma; however, recently, IFN monotherapy or IFN and ribavirin combination therapy have been reported to be effective.[93–96] Vallisa et al.[96] reported that administration of both pegylated IFN and ribavirin to 13 patients with HCV-associated non-Hodgkin B-cell lymphoma achieved a complete response in seven of these patients. It is interesting that IFN-based antiviral therapy has been demonstrated to be useful for malignant lymphoma associated with HCV-associated non-Hodgkin B-cell lymphoma in addition to conventional chemotherapy.

Autoimmune thyroid disease

The relationship between HCV infection and thyroid disease has been analyzed in many studies,[97,100] and a causal relationship between HCV infection and autoimmune thyroid disease has been particularly suggested.[98–100] Antonelli et al.[98] assessed the incidence of thyroid dysfunction in 630 chronic hepatitis C patients without cirrhosis or hepatocellular carcinoma who had not been treated with IFN by recruiting 389 patients from an iodine-deficient area, 268 patients from an iodine-sufficient area, and 86 patients with chronic hepatitis B aged 40 years or older as study subjects. The chronic hepatitis C patients exhibited a higher thyroid-

stimulating hormone level and lower free thyroxine and triiodothyronine levels than the controls. In addition, the chronic hepatitis C patients exhibited hypothyroidism and tended to have antithyroglobulin antibodies and anti-thyroid peroxidase antibodies. These findings suggest a relationship between HCV infection and thyroid disorder.[98] A possible relationship between HCV infection and thyroid cancer has also attracted attention recently.[99] The mechanism underlying the pathogenesis of thyroid disease associated with HCV infection has not yet been elucidated, but a relationship with liver/kidney microsomal antibody type 1 has been suggested.[99] Many patients with thyroid disorder caused by HCV infection are asymptomatic, requiring no special treatment. Thyroid disorder is also known to be an adverse reaction to IFN-α therapy for chronic hepatitis C.[99,101-104] Thyroid hypofunction caused by the administration of IFN-α is usually transient, and the patient recovers spontaneously after the end of the therapy. Hence, discontinuation of IFN-α is not required in many cases.[103]

Idiopathic interstitial pneumonitis

Recently, viral infection has been suggested to be a cause of idiopathic interstitial pneumonitis.[104] With regard to the relationship between HCV infection and idiopathic interstitial pneumonitis, Ueda et al.[106] reported in 1992 that the anti-HCV antibody positivity rate in 66 patients with idiopathic interstitial pneumonitis determined by enzyme-linked immunosorbent assay was 28.8%, which was significantly higher than that in 9464 normal subjects serving as controls.[106] It has not yet been clarified how HCV infection is associated with the pathogenesis of idiopathic interstitial pneumonitis. Kubo et al.[107] suggested that activated T lymphocytes and eosinophils are related to the pathogenesis of idiopathic interstitial pneumonitis associated with HCV infection, because they observed increased activated T-lymphocyte and eosinophil counts in the bronchoalveolar fluid of 13 chronic hepatitis C patients, despite their having the same total cell counts as normal subjects. On the other hand, studies disagree regarding the relationship between HCV infection and idiopathic interstitial pneumonitis,[108] and in-depth studies of this issue are expected. Idiopathic interstitial pneumonitis is also reported to be an adverse reaction to IFN therapy in chronic hepatitis C patients.[109] Such patients often have a high pretreatment KL-6 level, and the potential of their developing idiopathic interstitial pneumonitis is suggested. Recovery from IFN therapy-induced idiopathic interstitial pneumonitis is achieved by the discontinuation of the therapy,[109] but steroid administration is required in some cases.

Rheumatoid arthritis

HCV-associated rheumatoid arthritis complicated by cryoglobulinemia or Sjögren's syndrome has been reported.[10,39] For further information, please refer to the cited references.

Mooren's ulcer

Mooren's ulcer is a progressive ulcer associated with congestion and pain around the cornea.[110] HCV infection has been suggested to contribute to the development of this disease.[111-113] The effectiveness of IFN therapy for HCV-associated Mooren's ulcer has been reported,[110,111] but the exacerbation of ocular pain following the discontinuation of IFN therapy has also been observed; hence, caution is required.[111] Systemic corticoid administration has also been reported to be effective.[112] However, other investigators reported a negative correlation between HCV infection and Mooren's ulcer.[114-116] It is hoped that further detailed studies will clarify this issue.

Conclusion

It is necessary to consider possible complications associated with extrahepatic diseases in the treatment of HCV-infected patients.

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ELSEVIER

Impact of New Methicillin-Resistant *Staphylococcus aureus* Carriage Postoperatively After Living Donor Liver Transplantation

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ABSTRACT

Background. Preoperative carriage of methicillin-resistant *Staphylococcus aureus* (MRSA) is associated with an increased risk of MRSA infection after liver transplantation. It is not known, however, whether new MRSA carriage postoperatively also increases the risk of MRSA infection after liver transplantation.

Methods. We retrospectively reviewed the data from 242 adult patients who underwent living donor liver transplantation (LDLT) including microbiological and medical records from admission to 3 months after LDLT. Uni and multivariate analyses were performed to identify independent risk factors for postoperative MRSA infection among preoperative noncarriers of MRSA.

Results. Postoperative MRSA infection occurred in 18 of 219 preoperative noncarriers of MRSA by median postoperative day 26. Operation time of at least 16 hours and postoperative colonization with MRSA independently predicted postoperative MRSA infection.

Conclusion. Postoperative surveillance cultures should be performed periodically after liver transplantation to identify high-risk candidates for postoperative MRSA infection, even among preoperative noncarriers of MRSA.

STAPHYLOCOCCUS AUREUS is a major cause of bacterial infection after liver transplantation.[1,2] Isolates of *S aureus* causing clinical nosocomial infection can be divided into two groups: methicillin-susceptible *S aureus* and methicillin-resistant *S aureus* (MRSA). MRSA infection frequently complicates the postoperative course after deceased donor liver transplantation (DDLT).[1,3–5] Among several centers, 91% (45 of 49 isolates) of all *S aureus* infections after DDLT were caused by MRSA.[4]

Preoperative MRSA carriage is associated with an increased risk for MRSA infection after DDLT.[1,3–5] In addition, postoperative MRSA colonization is prevalent in DDLT.[6] Positive MRSA cultures on both postoperative and preoperative surveillance is considered important because increased MRSA colonization in a patient during hospitalization increases the risk of MRSA infection.[7] In a prospective study,[7] the relative risk of developing an MRSA infection among patients with MRSA colonization was greater than among patients who were not colonized with *S aureus*. In this particular study, 12 of 394 patients had MRSA colonization during hospitalization, and 4 of the 12 later developed MRSA infection.

It is not known, however, whether new MRSA carriage postoperatively following liver transplantation also increases the risk of MRSA infection. Moreover, MRSA in cases of living donor liver transplantation (LDLT), in which operations are scheduled in a more selective manner, is not well described. The aim of the present study was to assess the details of postoperative MRSA infection among preoperative noncarriers of MRSA and to analyze whether new MRSA carriage postoperatively increased the risk of MRSA infection after LDLT using multivariate analysis.

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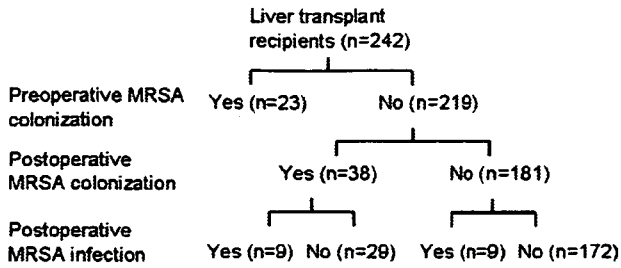

Fig 1. The patient profile of postoperative MRSA colonization and infection. MRSA, methicillin-resistant *S aureus*.

PATIENTS AND METHODS

Patients

We reviewed the 242 patients who underwent LDLT between 1996 and 2004, including 23 colonized with MRSA preoperatively, who were excluded from the study. Of the remaining patients, 119 were men and 100 were women of median age 50 years (range, 19 to 67). The indications included hepatitis C (n = 62), followed by primary biliary cirrhosis (n = 48) and hepatitis B (n = 31). The median Child-Pugh and model for end-stage liver disease (MELD) scores of those patients were 10 (range, 5 to 14) and 13 (range, −3 to 48), respectively. Our donor selection criteria[8] and surgical techniques for recipient and donor operations have been described elsewhere.[9]

Perioperative Management

Antimicrobial prophylaxis consisted of intravenous cefotaxim (1.0 g just before surgery, followed by 1.0 g every 6 hours intraoperatively and thereafter), ampicillin/sulbactam (1.0 g just before surgery, followed by 1.5 g every 12 hours intraoperatively and thereafter), and gentamicin, 60 mg every 12 hours after surgery) for 5 days. Fluconazole (200 mg every 24 hours) was administered intravenously for 7 days after surgery. All patients received the same immunosuppressive regimens using tacrolimus (Prograf, Fujisawa Pharmaceutical Corporation, Tokyo, Japan) and methylprednisolone (Solu-Medrol, Pfizer Inc, New York, NY, USA).[10]

Microbiological Data Collection

All patients were screened preoperatively for *S aureus* after admission for LDLT. Follow-up specimens were collected twice a week during the first month after LDLT and thereafter once a week during the hospital stay. Screened specimens consisted of swabs of the anterior nares, pharynx, sputum, urine, stool, swabs of wound or skin lesions, bile, and abdominal cavity discharge. A catheter or blood sample was also submitted when infection was suspected.

Specimens were plated onto mannitol-salt agar or sheep blood agar. *S aureus* was identified using standard microbiological methods. Methicillin resistance was determined using a disk diffusion test performed on Mueller-Hinton agar after incubation for 24 to 48 hours at 30°C. The strains with an oxacillin minimum inhibitory concentration value of at least 4 μg/mL were defined as MRSA colonization. Patients colonized with *S aureus* at any site and at any time during the hospital stay were considered carriers, and contact precautions were taken in cases with MRSA.

Definition of MRSA Infection

The medical and microbiological records of the patients were reviewed for the occurrence of MRSA infection in the 3 months following LDLT. Only the first MRSA infection was recorded for each patient.

Nosocomial infections were defined according to the reports from the Centers for Disease Control and Prevention in 1988 and in 1992, as described elsewhere.[11] Surgical site infection included superficial incisional, deep incisional, and organ/space infections that occurred within 30 days after surgery. Wound and intra-abdominal cavity infections that occurred more than 1 month after the operation were defined as a gastrointestinal system infection. When an organism isolated from blood culture was compatible with a related nosocomial infection at another site, the bloodstream infection was classified as a secondary bloodstream infection. When MRSA was isolated from culture samples in the presence of nosocomial infection including surgical site infection and other pathogenic organisms were absent, MRSA infection was diagnosed. An MRSA-positive culture sample without the presence of clinical symptoms was diagnosed as MRSA colonization.

Statistical Analysis

Background and clinical data collected for each patient included preoperative, surgical, and postoperative variables. Quantitative variables are presented as medians and ranges. Categorical variables are presented as absolute counts. Univariate analysis was used to identify associations between each of the variables and postoperative MRSA infection. Chi-square test or Fisher exact test was used to compare categorical data.

For multivariate analysis, only variables with a $P < .20$ in the univariate analysis were entered into a logistic regression model by the backward-elimination procedure. The final regression model included covariates associated with a likelihood ratio of $P < .1$. The results of the logistic regression were reported as odds ratios with 95% confidence intervals. A P value of less than .05 was considered statistically significant. All statistical analyses were performed using the JMP5.1 software package (SAS institute Inc, Cary, NC, USA).

RESULTS

Postoperative MRSA Colonization and Infection (Fig 1)

Postoperative MRSA infection occurred in 18 patients among the preoperative noncarriers of MRSA: nine patients were new MRSA carriers postoperatively, and nine

Table 1. Postoperative MRSA Infection in 18 Patients

	Colonized with MRSA (n = 9)	Noncarriers with MRSA (n = 9)	Total (n = 18)
Onset of MRSA infection (postoperative day)	16 (7–54)	40 (9–64)	26 (7–64)
Duration between colonization and infection	13 (2–21)	0	1 (0–21)
During hospitalization infection	9	8	17
SSI	6	3	9
Deep incisonal SSI	6	0	6
Organ/space SSI	0	3	3
Gastrointestinal system infection	2*	4	6
Intra-abdominal infection	2	4	6
Pneumonia	0	1	1
Lower respiratory infection	1	0	1
Primary BSI	0	1	1
Laboratory-confirmed BSI	0	1	1

*One patient had secondary surgical site infection. SSI, surgical site infection; BSI, bloodstream infection.

Table 2. Association Between Postoperative MRSA Infection and Preoperative, Surgical, and Postoperative Variables

Variables	MRSA Infection (−) (n = 201)	MRSA Infection (+) (n = 18)	P Value
Preoperative variables			
Age (y) ≥50	51 (19–67)	48 (24–62)	
	111	8	.46
Gender (male/female)	106/95	13/5	.14
Underlying liver disease			
Hepatitis C	55	7	
Primary biliary cirrhosis	46	2	
Hepatitis B	30	1	
Fulminant hepatitis	19	3	
Biliary atresia	10	1	
Autoimmune hepatitis	8	1	
Primary sclerosing cholangitis	8	1	
Metabolic disease	9	0	
Cryptogenic cirrhosis	6	0	
Alcoholic cirrhosis	2	2	
Others	8	0	
Hepatocellular carcinoma	59	5	1.0
Child-Pugh score	10 (5–14)	11 (5–12)	
≥10	105	13	.14
MELD score	12.9 (−3.4–48.2)	14.6 (4.3–29.4)	
≥15	67	6	1.0
Ascites	95	12	.14
Use of diuretics	109	12	.34
Encephalopathy	32	4	.51
Preoperative apheresis	38	4	.76
PT-INR	1.61 (0.89–7.48)	1.60 (1.23–2.35)	
≥1.7	80	6	.80
Serum bilirubin (mg/dL)	4.1 (0.3–38.6)	7.3 (1.2–32.4)	
>3.0	134	13	.80
Serum albumin (g/dL)	2.9 (1.5–4.4)	2.8 (1.8–3.8)	
>2.8	71	9	.31
Serum creatinine (mg/dL)	0.71 (0.2–7.7)	0.62 (0.4–2.4)	
≥1.5	11	2	.29
Steroid pulse therapy	23	2	1.0
Use of antimicrobials	46	8	.08
Beta lactam	37	7	.06
Glycopeptide	2	0	1.0
Fluroquinolone	13	3	.13
Amynoglycoside	5	1	.41
Others	2	0	1.0
History of abdominal surgery	93	8	1.0
Diabetus mellitus	24	2	1.0
MSSA colonization	100	10	.81
Surgical variables			
Operation time (h)	14.9 (10.7–33.2)	16.3 (12.2–19.3)	
≥16	64	11	.02
Blood loss (mL)	5240 (830–53835)	4415 (2590–34800)	
≥5000	106	8	.62
Blood transfusion (mL)	6970 (900–42890)	6385 (4240–26240)	
≥8000	83	6	.62
GV/SLV ratio (%)	46 (25–88)	42 (36–66)	
≥40	160	15	1.0
Duct to duct biliary reconstruction	144	14	.78
Postoperative variables			
ICU stay (d)	5 (3–46)	5 (4–26)	
≥10	18	4	.09
Apheresis	23	6	.02
Reoperation	72	4	.31
Acute rejection	58	5	1.0

Table 2. (continued)

Variables	MRSA Infection (−) (n = 201)	MRSA Infection (+) (n = 18)	P Value
Cytomegalovirus infection	87	5	.22
Fungal infection	6	1	.46
Colonization with MRSA	29	9	.001

PT-INR, the international normalized ratio of prothrombin time; MSSA, methicillin-susuceptible *S aureus*; MRSA, methicillin-resistant *S aureus*; GV, graft volume; SLV, standard liver volume; ICU, intensive care unit.

patients were MRSA noncarriers until the onset of infection. During the study period, 29 patients were asymptomatic carriers of MRSA. Among the nine patients who were colonized with MRSA postoperatively and subsequently developed infection, the MRSA-colonized sites before the onset of infection were sputum in six, stool in six, nares in five, pharynx in five, urine in two, discharge from an abdominal drain in two, and ascites in one patient.

Details of Postoperative MRSA Infection (Table 1)

The median days to onset of MRSA infection in all the patients with infection, in patients colonized with MRSA before infection, and in patients colonized concurrently with infection were postoperative days 26, 16, and 40, respectively. Among patients who were colonized with MRSA before infection, the median duration between the onset of colonization and infection was 13 days. During the study period, median length of hospital stay after LDLT was 50 (range, 6 to 90) days for patients without MRSA infection and 68 (range, 46 to 90) days for those with MRSA infection. MRSA infection occurred during hospitalization in 17 patients and after discharge in one patient.

Surgical site infection was detected in nine patients. One patient with gastrointestinal system infection had a secondary bloodstream infection. We treated MRSA infection with intravenous vancomycin in 12 patients, reoperation and intravenous vancomycin in two, reoperation alone in two, lavage of the intra-abdominal cavity through the surgical drain in one, and debridement of the wound in one. None of the 18 patients with MRSA infection died during the 3 months after LDLT.

Risk Factors for Postoperative MRSA Infection (Tables 2, 3)

Postoperative MRSA infection was significantly associated with operation time (≥16 hours; P = .02), postoperative apheresis (P = .02), and postoperative colonization with MRSA (P = .001, Table 2). In the multivariate analyses (Table 3), 10 risk factors with P values of less than .20 were entered into a logistic regression model using the backward-elimination procedure. In the final model, operation time (≥16 hours; odds ratio, 3.27) and postoperative colonization with MRSA (odds ratio, 7.13) independently predicted postoperative MRSA infection.

Table 3. Multivariate Analysis of Risk Factors for MRSA Infection After LDLT

Variable	Odds Ratio (95% Confidence Interval)	P Value
Preoperative use of beta lactam	3.03 (0.95–9.37)	.06
Operation time (h) ≥16	3.27 (1.15–9.89)	.03
Colonization with MRSA	7.13 (2.43–21.65)	.0004

MRSA, methicillin-resistant *S aureus*.

DISCUSSION

We have shown the impact of postoperative colonization with MRSA on subsequent MRSA infection after LDLT. Among patients with MRSA infection, 9 of 18 (50%) in the present study were colonized with MRSA before the onset of infection. MRSA infection occurred soon after the operation in patients who were new MRSA carriers postoperatively. Of 18 patients with MRSA infection, 10 developed the infection within 1 month after LDLT, among whom seven were colonized with MRSA before the onset of infection. In addition, patients who were colonized with MRSA developed MRSA infection soon after colonization with MRSA. Of nine patients with MRSA colonization and subsequent infection, all developed infections within 3 weeks after colonization with MRSA.

Postoperative surveillance cultures should be performed at multiple sites, including the nares, after LDLT. Although the anterior nares is the most frequent carriage site for *S aureus*,[12] other extranasal sites such as skin, perineum, pharynx, gastrointestinal tract, vagina, and axillae can also harbor the organism.[5,12] Among nine patients who were colonized with MRSA postoperatively and subsequently developed infection, nasal colonization was detected in 5 (56%). If surveillance culture is performed for only the nares as reported in previous studies in DDLT, new postoperative carriers of MRSA at sites other than the nares[1,6] might be overlooked, thereby delaying the administration of appropriate antimicrobials such as vancomycin in patients suspected of MRSA infection.

The results of the present study indicated that postoperative MRSA colonization and prolonged operative time independently increased the risk of postoperative MRSA infection. MRSA infection is well described in previous studies of DDLT.[1,3–5] Most studies have reported that preoperative MRSA carriage increased the risk of MRSA infection, but these studies[2,3,5] were not focused on the impact of new postoperative MRSA carriage on subsequent infection. Of 38 patients, 9 (24%) who were colonized with MRSA subsequently developed MRSA infection in the present study. This rate is comparable to that of the previous reports [around 30%].[7,13] In one recent retrospec-

tive study, 60 of 209 (29%) patients developed subsequent MRSA infection in the 18-month period after the initial MRSA-positive culture.[13] Postoperative surveillance culture should be performed periodically after LDLT to identify new MRSA carriers who are high-risk candidates for subsequent MRSA infection.

Prolonged operative time increased the risk of MRSA infection in the present study. Prolonged surgical duration indicates technically more difficult surgical procedures in which the risk of complication is increased.[14] George et al[15] used multivariate analysis to demonstrate that prolonged surgery duration increased the risk of bacterial infection among in liver transplant recipients. In contrast, Singh et al[1] reported that there was no such association.

Intense antimicrobial use, measured by the administration of preoperative antimicrobials, during the month before LDLT did not correlate with postoperative MRSA infection among preoperative noncarriers of MRSA in the present study. Although there is little doubt that widespread use of antimicrobials provides multidrug-resistant strains of MRSA with a selective survival advantage,[16] the relationship between MRSA and antimicrobials seemed more complex in the current series. Some studies using multivariate analysis have failed to show such an association.[17] In other studies, exposure to specific antimicrobials, such as third-generation cephalosporins, amoxicillin with clavulanic acid, quinolones, and broad-spectrum antibiotics, increased the risk of MRSA infection or colonization.[18] Crowcroft et al[19] found no association between total antimicrobial use and MRSA colonization or infection, suggesting that the problem was inappropriate rather than excessive use of antimicrobials. This discrepancy is probably due to the fact that in the present study, all patients received multiple antimicrobials, resulting in broad coverage as perioperative prophylaxis, per protocol, and it is difficult to detect the effect of a specific antimicrobial.

One limitation to the present study is that we could not differentiate specific MRSA strains. Pulsed-field gel electrophoresis analysis was not accessible. Therefore, we could not analyze the impact of MRSA transmission, such as patient-to-patient transmission by transient carriage on the hands of the medical staff. Similarly, it was not possible to determine whether infection was due to the same strain as that of the colonization or to a newly acquired strain when the infection occurred. Chang et al[4] analyzed isolates from infected sites and those from the anterior nares in seven patients with MRSA infection, reporting detection of the same isolates. Such a detailed analysis might yield further information to elucidate the relationship between new postoperative MRSA carriage and subsequent infection following LDLT.

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Hepatitis C virus core protein induces spontaneous and persistent activation of peroxisome proliferator-activated receptor α in transgenic mice: Implications for HCV-associated hepatocarcinogenesis

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Persistent infection of hepatitis C virus (HCV) can lead to a high risk for hepatocellular carcinoma (HCC). HCV core protein plays important roles in HCV-related hepatocarcinogenesis, because mice carrying the core protein exhibit multicentric HCCs without hepatic inflammation and fibrosis. However, the precise mechanism of hepatocarcinogenesis in these transgenic mice remains unclear. To evaluate whether the core protein modulates hepatocyte proliferation and apoptosis *in vivo*, we examined these parameters in 9- and 22-month-old transgenic mice. Although the numbers of apoptotic hepatocytes and hepatic caspase 3 activities were similar between transgenic and nontransgenic mice, the numbers of proliferating hepatocytes and the levels of numerous proteins such as cyclin D1, cyclin-dependent kinase 4 and c-Myc, were markedly increased in an age-dependent manner in the transgenic mice. This increase was correlated with the activation of peroxisome proliferator-activated receptor α (PPARα). In these transgenic mice, spontaneous and persistent PPARα activation occurred heterogeneously, which was different from that observed in mice treated with clofibrate, a potent peroxisome proliferator. We further demonstrated that stabilization of PPARα through a possible interaction with HCV core protein and an increase in nonesterified fatty acids, which may serve as endogenous PPARα ligands, in hepatocyte nuclei contributed to the core protein-specific PPARα activation. In conclusion, these results offer the first suggestion that HCV core protein induces spontaneous, persistent, age-dependent and heterogeneous activation of PPARα in transgenic mice, which may contribute to the age-dependent and multicentric hepatocarcinogenesis mediated by the core protein.

Key words: cell-cycle regulator; peroxisome; nuclear stabilization; heterogeneous PPARα activation

Hepatitis C virus (HCV) is one of the major causes of chronic hepatitis, and persistent infection with this virus can lead to a high incidence of hepatocellular carcinoma (HCC).[1,2] The prevalence of HCC because of chronic HCV infection has increased over the past two decades,[3,4] and chronic HCV infection has therefore been recognized as a serious disease. However, the precise mechanism of hepatocarcinogenesis during chronic HCV infection remains unclear.

Many experiments using cell culture systems have suggested the possibility that HCV core protein itself can modulate various cellular functions and can be directly linked to the development of HCV-related HCC.[5] For example, HCV core protein transforms rat embryo fibroblasts to a tumorigenic phenotype in cooperation with the H-*ras* oncogene,[6] suppresses *c-myc*-related apoptosis[7] and transcription of the *p53* gene,[8] interacts with a variety of proteins, including helicase, lymphotoxin-β receptor, or dead box protein, and modulates their functions.[9] We further established transgenic mouse lines carrying the HCV core gene, in which the core protein is constitutively expressed in the liver at levels similar to that found in chronic hepatitis C patients.[10] These mice exhibited multicentric hepatic adenomas, and developed HCCs in an age-dependent manner.[11] The livers of these mice were almost free of inflammation, necrosis and fibrosis,[10,11] suggesting that the core protein itself has a hepatocarcinogenic potential *in vivo*. However, the molecular mechanism of the development of HCC in the transgenic mice has not been fully understood.

In the livers of HCV core gene transgenic mice, an age-dependent increase in oxidative stress and resultant DNA damage were found,[12] and these effects may contribute to or facilitate the development of HCC. Another possible mechanism of hepatocarcinogenesis is continuous enhancement of hepatocyte proliferation. Cell proliferation and apoptosis are highly regulated processes for maintaining homeostasis in many organs, and during the carcinogenic process, sustained imbalance generally precedes cancer.[13,14] For example, in patients with chronic HCV infection, high hepatocyte proliferative activity relative to apoptosis may reliably predict a new development of HCC.[15] However, there is no information about whether or not hepatocyte proliferation accelerates persistently in mice carrying the HCV core gene, and no information about how the core protein promotes hepatocyte proliferation *in vivo*. In the current study, we began to examine changes in the parameters of hepatocyte proliferation and apoptosis in the transgenic mice.

Material and methods

Animals and treatments

HCV core gene transgenic mice on a C57BL/6N genetic background were produced as described earlier.[10] Because HCC developed preferentially in male transgenic mice,[11] 9- and 22-month-old male mice ($n = 8$ for either age group) were adopted. Sex- and age-matched nontransgenic mice ($n = 8$ for either age group) were used as controls. These mice were fed an ordinary diet and were treated in a specific pathogen-free state according to the institutional guidelines. For additional experiment, male wild-type mice fed a control diet containing 0.5% clofibrate for 2 weeks ($n = 8$) were used. All mice were killed by cervical dislocation and the livers were excised. When a hepatic tumor was present, it was removed and the remaining liver tissue was used. All experiments were performed in accordance with animal study protocols approved by the Shinshu University School of Medicine.

Abbreviations: AOX, acyl-CoA oxidase; CDK, cyclin-dependent kinase; DAB, 3,3'-diaminobenzidine; FITC, fluorescein isothiocyanate; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; L-FABP, liver-type fatty acid-binding protein; NEFA, nonesterified fatty acid; PBS, phosphate-buffered saline; PCNA, proliferating cell nuclear antigen; PMSF, phenylmethylsulfonyl fluoride; PPAR, peroxisome proliferator-activated receptor; PT, peroxisomal thiolase; RXR, retinoid X receptor; SDS, sodium dodecyl sulfate; TUNEL, terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling.

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