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RAPID COMMUNICATION

Reduction of virus burden-induced splenectomy in patients with liver cirrhosis related to hepatitis C virus infection

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Abstract

AIM: To examine the hepatitis C virus (HCV) levels and immunological markers in cirrhotic patients after splenectomy.

METHODS: HCV RNA titers as well as cellular and humoral immune markers were determined in 20 cirrhotic patients after splenectomy and in 32 cirrhotic controls with an intact spleen.

RESULTS: Serum HCV RNA titers were lower in the splenectomized patients than in the controls ($186 \pm 225 \times 10^3$ copies/mL *vs* $541 \pm 417 \times 10^3$ copies/mL, $P < 0.01$). HCV RNA was judged to have been spontaneously eradicated in 4 splenectomized patients, but in none of the controls. Natural killer cell activity was higher in the splenectomized patients than in the controls (41.2 ± 19.3% *vs* 24.7 ± 15.3%, $P < 0.01$), and natural killer cell activity was negatively correlated to HCV RNA titers in the splenectomized patients except in those with serotype 2-related infection. The CD4/CD8 ratio was significantly lower in the splenectomized patients than in the controls.

CONCLUSION: The findings suggest that splenectomy may diminish virus burden in cirrhotic patients with HCV infection at least in part, through augmentation of natural killer cell activity.

Key words: Hepatitis C virus; Liver cirrhosis; Natural killer cell; Splenectomy

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INTRODUCTION

Persistent infection with hepatitis C virus (HCV), a parenterally transmitted RNA virus, occurs in 70%-80% of HCV-infected patients. Chronic hepatitis C progresses gradually to liver cirrhosis, a condition frequently associated with hepatocellular carcinoma (HCC)[1-3]. To prevent progression to chronic liver disease, continuous virus burden must be interrupted. However, spontaneous elimination of the virus load rarely occurs[4-8]. HCV-specific cytotoxic T cells are thought to play a principal effector role in host defense against HCV infection[9-11]. In addition, natural killer (NK) cells are believed to participate in the defense against hepatitis viruses, because the human liver contains a significantly higher number of NK cells than peripheral blood or any other organs[12]. NK cells exert various effector functions during the early phase of HCV infection, including induction of apoptosis and production of IFN-gamma and TNF-alpha[13-16]. In addition, NK cells are suggested to play a crucial role in the clearance of HCV in patients undergoing interferon therapy[17,18], but the significance of NK cells in the pathogenesis of chronic hepatitis C remains to be clarified. Although several studies have reported reduced activity of NK cells in cirrhotic patients with a history of alcohol abuse[19,20] and complication of HCC[21-23], little is known whether NK cells vary in patients with HCV-positive liver cirrhosis[24,25].

Splenectomy was a popular surgical procedure for esophageal varices associated with liver cirrhosis until endoscopic sclerotherapy has become the first line treatment[26,27]. Splenectomy may compromise the immune system because the spleen plays an important role in phagocytosis and antibody production[28,29]. On the other hand, there is some evidence that splenectomy can positively affect Kupffer cell functions in the liver, and protects against viral infection[30-33]. Ferrante *et al*[32] have insisted that a compensatory increase in the activity of NK cells in splenectomized patients might offer protection against infection and malignant disease. Pereira *et al*[31] reported that the HCV genome is detectable in spleen specimens obtained from HCV antibody seropositive patients associated with chronic schistosomiasis, suggesting that the spleen is an extrahepatic reservoir of the virus. Splenectomy may therefore influence the immune system and virus load of cirrhotic patients with HCV infection. Changes in immune

mediators and HCV burden in cirrhotic patients after splenectomy remain to be evaluated. In the present study, we retrospectively evaluated the effect of splenectomy on replication of HCV as well as cellular and humoral immunity including NK cell activity and lymphocyte subsets in cirrhotic patients with HCV infection.

MATERIALS AND METHODS

Patients

Twenty Japanese cirrhotic patients positive for anti-HCV antibody after splenectomy were enrolled in the present study (splenectomized). Splenectomy was performed in combination with surgical treatment of esophageal varices (14 cases), HCC (5 cases), or gastric cancer (1 case). The patients were referred for follow-up at our hospital. The mean duration of follow-up after splenectomy was 9.2 years. Thirty-two HCV-positive cirrhotic patients with an intact spleen served as controls and were followed up at our hospital. All controls had complications of esophageal varices, 17 of whom were treated for esophageal varices with endoscopic sclerotherapy and/or band ligation. Morphological diagnosis of post-hepatitic cirrhosis was performed in all splenectomies and 22 controls. The remaining 10 controls were diagnosed clinically based on typical clinical and laboratory findings of liver cirrhosis and characteristic liver findings at computed tomography and ultrasonography, because 6 patients showed complicated severe coagulopathy and 4 patients refused liver biopsy. No patients were diagnosed with chronic liver diseases, such as alcoholic hepatitis, autoimmune hepatitis, and chronic hepatitis B infection, or drug-induced liver disease. Patients with a history of alcohol abuse or diagnosed as HIV positive were excluded. None of our patients underwent anti-viral therapy using interferon during the period studied. Patient characteristics are shown in Table 1. Clinical features, severity of liver cirrhosis according to Child' s classification, association of HCC, HCV serotype, and liver function tests did not differ significantly between the two groups. Platelet counts were significantly higher in the splenectomized patients than in the controls. The study protocol was approved by the Ethics Committee of the Department of Internal Medicine, and informed consent was obtained from all patients.

Serum HCV RNA levels were measured to evaluate the effect of splenectomy on viral load. In addition, peripheral activity of NK cells, proportion of CD4 and CD8 subsets of T cells, and levels of serum β2-microglobulin and soluble interleukin 2 receptor (sIL2R) were examined.

Virology

The presence of HCV antibody was determined by the second and/or third generation of enzyme-linked immunosorbent assay (Ortho Diagnostic System Co., Ltd., Tokyo, Japan) and confirmed by recombinant immunoblot assay (Chiron RIBA-2 and/or RIBA-3). HCV serotype was examined by serotyping assay (SRL Laboratory Co., Tokyo, Japan) according to the method of Tsukiyama-Kohara *et al*[34]. Serotype 1 corresponded to types 1a and 1b, while serotype 2 corresponded to types 2a and 2b of the Simmonds classification[35]. Quantitative levels of HCV-RNA in serum samples were analyzed by combined reverse transcription PCR assay (Amplicor-HCV monitor; Nippon Roche, Tokyo, Japan) that could detect viral concentrations above 10^3 copies per mL[36]. If serum HCV-RNA was undetectable by this assay, we performed the Amplicor hepatitis C viral test twice, which is more sensitive and can detect as low as 10^2 copies per mL[37]. If HCV RNA was still undetectable, it was judged to indicate 'virus eradication'.

Table 1 Characteristics of cirrhotic patients with HCV infection

	Splenectomy (+)	Splenectomy (-)
Age (yr)	62.6±5.5	63.2±13.6
Gender (m:f)	8:12	13:19
HCV Serotype		
1	15	29
2	3	3
unknown[a]	2	0
Child's Classification		
A	11	18
B	6	8
C	3	6
Association of HCC	7/20	9/32
Total bilirubin (mg/dL)	1.2±0.5	1.2±0.7
Albumin (g/L)	35±6	37±4
γ-globulin (g/L)	23±0.8	20±5
AST (IU/L)	65.3±30.5	61.8±23.7
ALT (IU/L)	31.5±9.1	25.1±4.9
Platelets ($\times 10^4$ /μL)	15.2±4.5	8.1±2.7[b]
NK cell activity (%)	41.2±19.3	24.7±15.3[b]
CD4/CD8	1.2±0.4	1.6±0.6[c]
β2-MG (mg/L)	2.5±1.2	2.4±0.6[n.s]
sIL2R (U/mL)	917±445	842±190[n.s]

HCC: hepatocellular carcinoma; AST: aspartic aminotransferase; ALT: alanine aminotransferase; NK cells: natural killer cells; β2-MG: β2-microglobulin; sIL2R: soluble interleukin 2 receptor. a: undeterminable serotype; [b]$P<0.001$, [c]$P<0.05$ *vs* splenectomized patients; NS: no significant difference.

Immunological markers

NK cell activity was assessed against the k-562 cell line (Dainippon Pharmaceutical Co., Osaka, Japan) marked with ^{51}Cr using a cytotoxicity test for 3.5 h[38]. Blood samples taken from the cubital vein were collected into heparinized tubes. After centrifugation of the blood sample with a lymphocyte separation medium (Limphosepar I; Tokyo, Japan), the interface mononuclear cells were collected and suspended at a cell density of 1×10^6 /mL in RPMI-1640 medium (IBL; Gunma, Japan) and supplemented with 10% FBS (Cansera; Ontario, Canada). Peripheral blood monocytes (2×10^5 cells) were added to round-bottomed 96-well microplates containing ^{51}Cr-labeled target cells (1×10^4 cells) in 0.2 mL of RPMI-1640 medium supplemented with 10% FBS. The effector cell/target cell ratio was determined as 20. After centrifugation at 800 r/min for 5 min using an exclusive centrifuge for microplates, the cells were incubated for 3.5 h at 37℃ under 50 mL/L CO_2 in air. After incubation, the culture supernatant was harvested using PETΣ-96 (Sohken; Tokyo, Japan), and the radioactivity was determined using a gamma counter (1272 clini gamma, Wallac; Turuku, Finland). The percentage of cytotoxicity was calculated as follows: % cytotoxicity = (experimental ^{51}Cr release - spontaneous ^{51}Cr release) /(maximal ^{51}Cr release-spontaneous ^{51}Cr release) × 100.

The proportion of peripheral CD4 and CD8 subsets

Table 2 Characteristics of cirrhotic patients after splenectomy

	Age	Sex	Years After splenectomy	Serotype	HCV RNA (k copies/mL)	Child's classification	association of HCC[1]
1	75	Female	19	1	23	C	no
2	60	Female	16	Unknown[2]	Negative	A	no
3	80	Female	15	1	140	A	no
4	70	Female	15	1	130	B	no
5	63	Male	14	1	2	B	yes
6	67	Female	12	1	460	A	no
7	62	Male	12	1	Negative	A	no
8	50	Male	12	1	Negative	A	no
9	73	Female	11	Unknown[2]	Negative	A	no
10	67	Female	11	2	<1	B	no
11	50	Male	10	1	480	C	no
12	60	Female	7	1	140	B	yes
13	56	Female	7	1	160	C	yes
14	38	Male	6	1	770	A	no
15	68	Male	5	1	210	B	yes
16	73	Female	2	1	510	A	no
17	66	Female	2	2	21	A	no
18	59	Female	2	1	420	A	yes
19	58	Male	1	2	14	B	yes
20	57	Male	1	1	380	A	yes

[1] Hepatocellular carcinoma; [2] undeterminable serotype.

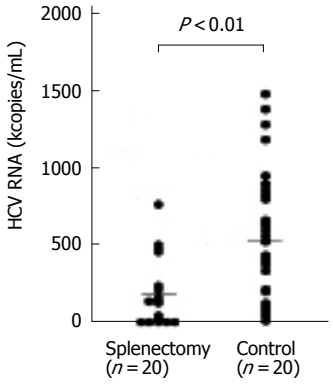

Figure 1 Serum HCV RNA titers in patients with liver cirrhosis. Splenectomized patients had significantly lower titers of HCV RNA than cirrhotic controls with an intact spleen.

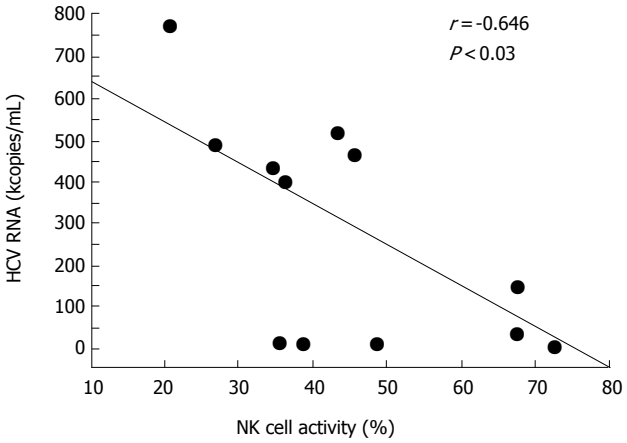

Figure 2 Relationship between HCV RNA titers and NK cell activity in non-serotype 2 splenectomized patients. There was a significantly negative correlation between HCV RNA titers and NK cell activity in non-serotype 2 splenectomized patients.

was assayed by flow cytometry. β2-microglobulin levels were measured by latex immunoaggregation assay (Eiken Co., Tokyo, Japan). sIL2R levels were determined using the cell-free IL-2R EIA kit.

Statistical analysis

Results were expressed as mean ± SD. Statistical analyses were carried out using the computer program StatView-J4.5 (Abacus Concepts, Inc. Berkeley, USA). Mean quantitative values were compared using Student's *t* test. Nonparametric data were compared using the Mann-Whitney U-test. All *p*-values were two-tailed. $P < 0.05$ was considered statistically significant.

RESULTS

HCV RNA titers (Figure 1)

Mean titers of HCV RNA were significantly lower in the splenectomized patients than in the cirrhotic controls ($186 \pm 225 \times 10^3$ copies/mL *vs* $541 \pm 417 \times 10^3$ copies/mL, $P < 0.01$). Among the serotype 1-related patients (15 splenectomized and 29 controls), the splenectomized patients showed a reduced viral load compared with the controls ($246 \pm 231 \times 10^3$ copies/mL *vs* $590 \pm 407 \times 10^3$ copies/mL, $P < 0.01$). Four (20.0%) of 20 splenectomized patients showed eradicated HCV in serum after 11 years of splenectomy in one patient, 12 years in two patients and 16 years in one patient. Two of the 4 patients had serotype 1-related HCV infection, while the serotype was undetermined in the other 2 patients (Table 2). No controls spontaneously eradicated HCV in their serum. Among the splenectomized patients with serotype 1, HCV RNA levels were lower, but not significantly lower in patients with a longer follow-up time (≥10 years) than in those with a shorter follow-up time (<9 years; $155 \pm 202 \times 10^3$ copies/mL *vs* $350 \pm 531 \times 10^3$ copies/mL, $P = 0.103$). No gender-related difference in virus load was observed in controls with serotype 1 (12 males: $649 \pm 469 \times 10^3$ copies/mL *vs* 17 females: $521 \pm 329 \times 10^3$ copies/ mL, $P = 0.43$).

Immunological markers (Table 1)

Peripheral activity of NK cells was significantly higher in the splenectomized patients than in the controls. In both splenectomized patients and controls, NK cell activity was lower in patients complicated by HCC than in those without HCC, although the difference was not significant [HCC (+): 33.6%±11.8%, HCC (-): 47.6%±18.1%, $P=0.16$]. NK cell activity in LC patients with an intact spleen was not significantly different from that in healthy subjects (data not shown).

The splenectomized patients showed a reduced proportion of CD4 cells, a similar proportion of CD8 cells and a significantly lower ratio of CD4/CD8 compared with those of the controls. Serum sIL2R and β2-microglobulin values were similar in both groups.

Relationship between HCV RNA levels and immunological markers

In the splenectomized patients without serotype 2-related infection, there was a significantly negative correlation between HCV RNA titers and NK cell activity (Figure 2). Such a relationship was not found in the controls. No relationships were found between HCV RNA levels and CD4/CD8 ratio, sIL2R, or β2-microglobulin.

DISCUSSION

This was the first retrospective study to examine the effect of splenectomy on the reduction of HCV in cirrhotic patients. We found that HCV RNA titers were significantly lower in cirrhotic patients after splenectomy than in cirrhotic controls with an intact spleen, indicating that splenectomy can reduce virus burden. The mechanisms through which splenectomy helps to reduce HCV remain to be determined, but a few possibilities have been suggested. One explanation for reduced viral load after splenectomy is that it might be immunologically based. The role of NK cells in controlling HCV replication remains obscure, but NK cells hold the potential to play a vital role in controlling HCV replication in hepatic cells using IFN-gamma-dependent mechanism[13]. Reports of the effect of splenectomy on the peripheral activity of NK cells are contradictory[27-31]. In the present study, HCV-positive cirrhotic patients splenectomy had the augmented activity of NK cells compared with those with an intact spleen. An inverse correlation between HCV RNA titers and NK cell activity was shown in the splenectomized patients, but not in the controls with an intact spleen. In addition, Ikuta *et al*[39] reported that liver mononuclear cells from splectomized mice produce a significantly larger amount of IFN-gamma than those from sham-operated mice. Taken together, increased NK cell activity post-splenectomy may reduce virus burden in HCV-positive cirrhotic patients.

The association of cirrhosis with HCC[21-23], cholestasis[40], and protein calorie malnutrition[20] is also important because these factors may influence the peripheral activity of NK cells in cirrhotic patients. Similar to previous reports, our data showed that cirrhotic patients complicated by HCC had a lower NK cell activity than those without HCC. The incidence of complication of HCC was slightly but not significantly higher in the splenectomized patients (35%) than in the controls (28%). Total bilirubin levels and severity of liver cirrhosis according to Child's classification were similar between the splenectomized patients and the controls. Accordingly, these biochemical and physiological factors did not cause increased activity of NK cells in patients after splenectomy.

Splenectomized patients with a longer follow-up time (≥10 years) had lower HCV RNA levels than those with a shorter follow-up time (9< years), although the difference was not significant. Further research should attempt to determine whether the lower HCV titers of patients with a longer follow-up time are due to the natural course of infection. To estimate the relevance of aging to virus load, we examined the changes in HCV levels over a mean period of 4.5 years in 20 patients with HCV-positive cirrhosis. The mean HCV RNA titers did not vary significantly during this period (baseline, 6.6±9.2 Meq/mL; after 4.5 years, 6.1±8.5 Meq/mL). Kato *et al*[41] reported that the amount of HCV RNA tends to increase as the duration of infection increases. These findings indicate that the virus load of splenectomized patients does not spontaneously reduce with age.

Interestingly, four splenectomized patients showed spontaneous eradication of HCV, whereas cirrhotic controls showed no such spontaneous clearance during the observation period. This is in contrast to the findings that spontaneous elimination of virus load rarely occurs in patients with persistent HCV infection and chronic liver disease[4-8]. To date, little attention has been focused on identifying the viral and host factors involved in the spontaneous disappearance of serum HCV RNA, because the time point of infection is unknown for many patients, which makes a prospective evaluation of long-term outcome of infection problematic. Evidence for factors related to the natural disappearance of HCV has been reported by Furuzaki *et al*[8]. HCV levels below 1.0 Meq/mL can contribute to natural clearance of the virus. Thus, reduced virus load caused by activated NK cells may have led to HCV eradication in four of the splenectomized patients.

The present findings suggest that splenectomy may diminish or remit virus burden in HCV-positive cirrhotic patients at least in part by increasing NK cell activity. However, simply because four HCV-eradicated patients underwent splenectomy before a method for HCV RNA assay was developed, it would be rash to conclude that splenectomy can lead to their clearance of HCV. Furthermore, influence of surgery for esophageal varices and HCC on virus load cannot be ruled out. A prospective study on the effect of splenectomy on HCV replication should be undertaken in order to clarify whether splenectomy may reduce virus burden and/or eradicate HCV in serum of HCV-positive cirrhosis.

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REFERENCES

1 **Seeff LB.** Natural history of chronic hepatitis C. *Hepatology*

2002; **36**: S35-S46
2 **Ikeda K**, Saitoh S, Suzuki Y, Kobayashi M, Tsubota A, Koida I, Arase Y, Fukuda M, Chayama K, Murashima N, Kumada H. Disease progression and hepatocellular carcinogenesis in patients with chronic viral hepatitis: a prospective observation of 2215 patients. *J Hepato*l 1998; **28**: 930-938
3 **Kiyosawa K**, Sodeyama T, Tanaka E, Gibo Y, Yoshizawa K, Nakano Y, Furuta S, Akahane Y, Nishioka K, Purcell RH. Interrelationship of blood transfusion, non-A, non-B hepatitis and hepatocellular carcinoma: analysis by detection of antibody to hepatitis C virus. *Hepatology* 1990; **12**: 671-675
4 **Okuda K,** Yokosuka O. Natural history of chronic hepatitis C in patients on hemodialysis: case control study with 4-23 years of follow-up. *World J Gastroenterol* 2004; **10**: 2209-2212
5 **Hattori Y,** Orito E, Ohno T, Sugauchi F, Suzuki S, Sugiura M, Suzumori K, Hattori K, Ueda R, Mizokami M. Loss of hepatitis C virus RNA after parturition in female patients with chronic HCV infection. *J Med Virol* 2003; **71**: 205-211
6 **Watanabe H,** Saito T, Shinzawa H, Okumoto K, Hattori E, Adachi T, Takeda T, Sugahara K, Ito JI, Saito K, Togashi H, Suzuki R, Hayashi M, Miyamura T, Matsuura Y, Kawata S. Spontaneous elimination of serum hepatitis C virus (HCV) RNA in chronic HCV carriers: a population-based cohort study. *J Med Virol* 2003; **71**: 56-61
7 **Kondili LA,** Chionne P, Costantino A, Villano U, Lo Noce C, Pannozzo F, Mele A, Giampaoli S, Rapicetta M. Infection rate and spontaneous seroreversion of anti-hepatitis C virus during the natural course of hepatitis C virus infection in the general population. *Gut* 2002; **50**: 693-696
8 **Fukuizumi K**, Sata M, Suzuki H, Kumashiro R, Tanikawa K. Natural disappearance of serum HCV RNA: prospective study in a hyperendemic area. *Hepatol Res* 1997; **9**: 144-151
9 **Rosen HR.** Hepatitis C pathogenesis: mechanisms of viral clearance and liver injury. *Liver Transpl* 2003; **9**: S35-S43
10 **Liaw YF,** Lee CS, Tsai SL, Liaw BW, Chen TC, Sheen IS, Chu CM. T-cell--mediated autologous hepatocytotoxicity in patients with chronic hepatitis C virus infection. *Hepatology* 1995; **22**: 1368-1373
11 **Bowen DG**, Walker CM. Adaptive immune responses in acute and chronic hepatitis C virus infection. *Nature* 2005; **436**: 946-952
12 **Doherty DG,** Norris S, Madrigal-Estebas L, McEntee G, Traynor O, Hegarty JE, O'Farrelly C. The human liver contains multiple populations of NK cells, T cells, and CD3+CD56+ natural T cells with distinct cytotoxic activities and Th1, Th2, and Th0 cytokine secretion patterns. *J Immunol* 1999; **163**: 2314-2321
13 **Li Y, Zhang T**, Ho C, Orange JS, Douglas SD, Ho WZ. Natural killer cells inhibit hepatitis C virus expression. *J Leukoc Biol* 2004; **76**: 1171-1179
14 **Tseng CT,** Klimpel GR. Binding of the hepatitis C virus envelope protein E2 to CD81 inhibits natural killer cell functions. *J Exp Med* 2002; **195**: 43-49
15 **Ortaldo JR**, Bere EW, Hodge D, Young HA. Activating Ly-49 NK receptors: central role in cytokine and chemokine production. *J Immunol* 2001; **166**: 4994-4999
16 **Biron CA,** Brossay L. NK cells and NKT cells in innate defense against viral infections. *Curr Opin Immunol* 2001; **13**: 458-464
17 **Lin AW**, Gonzalez SA, Cunningham-Rundles S, Dorante G, Marshall S, Tignor A, Ha C, Jacobson IM, Talal AH. CD56(+dim) and CD56(+bright) cell activation and apoptosis in hepatitis C virus infection. *Clin Exp Immunol* 2004; **137**: 408-416
18 **Van Thiel DH,** Zhang X, Baddour N, Wright HI, Friedlander L, Gavaler JS. Intrahepatic mononuclear cell populations and MHC antigen expression in patients with chronic hepatitis C [correction of B]: effect of interferon-alpha. *Dig Dis Sci* 1994; **39**: 970-976
19 **Laso FJ,** Madruga JI, Girón JA, López A, Ciudad J, San Miguel JF, Alvarez-Mon M, Orfao A. Decreased natural killer cytotoxic activity in chronic alcoholism is associated with alcohol liver disease but not active ethanol consumption. *Hepatology* 1997; **25**: 1096-1100
20 **Ledesma F,** Echevarria S, Casafont F, Lozano JL, Pons-Romero F. Natural killer cell activity in alcoholic cirrhosis: influence of nutrition. *Eur J Clin Nutr* 1990; **44**: 733-740
21 **Taketomi A,** Shimada M, Shirabe K, Kajiyama K, Gion T, Sugimachi K. Natural killer cell activity in patients with hepatocellular carcinoma: a new prognostic indicator after hepatectomy. *Cancer* 1998; **83**: 58-63
22 **Chuang WL,** Liu HW, Chang WY. Natural killer cell activity in patients with hepatocellular carcinoma relative to early development and tumor invasion. *Cancer* 1990; **65**: 926-930
23 **Kakumu S**, Hirofuji H, Fuji A, Tahara H, Yoshioka K. Phenotypic expression of natural killer cell associated membrane antigens in patients with chronic liver disease and hepatocellular carcinoma. *J Clin Lab Immunol* 1988; **26**: 29-32
24 **Takegoshi K,** Ogai H, Sugimoto T, Ohmoto Y. Production of IFN-gamma and IL-12 by peripheral whole blood is maintained in hepatitis C virus patients with persistently normal alanine transferase activity; A preliminary report. *Hepatol Res* 2004; **29**: 81-88
25 **Kawarabayashi N**, Seki S, Hatsuse K, Ohkawa T, Koike Y, Aihara T, Habu Y, Nakagawa R, Ami K, Hiraide H, Mochizuki H. Decrease of CD56(+)T cells and natural killer cells in cirrhotic livers with hepatitis C may be involved in their susceptibility to hepatocellular carcinoma. *Hepatology* 2000; **32**: 962-969
26 **Sugiura M**, Futagawa S. A new technique for treating esophageal varices. *J Thorac Cardiovasc Surg* 1973; **66**: 677-685
27 **Weese JL**, Starling JR, Yale CE. Control of bleeding esophageal varices by transabdominal esophageal transection, gastric devascularization, and splenectomy. *Surg Gastroenterol* 1984; **3**: 31-36
28 **Samimi F,** Irish WD, Eghtesad B, Demetris AJ, Starzl TE, Fung JJ. Role of splenectomy in human liver transplantation under modern-day immunosuppression. *Dig Dis Sci* 1998; **43**: 1931-1937
29 **Linet MS,** Nyrén O, Gridley G, Adami HO, Buckland JD, McLaughlin JK, Fraumeni JF Jr. Causes of death among patients surviving at least one year following splenectomy. *Am J Surg* 1996; **172**: 320-323
30 **Joag SV**, Stephens EB, Adams RJ, Foresman L, Narayan O. Pathogenesis of SIVmac infection in Chinese and Indian rhesus macaques: effects of splenectomy on virus burden. *Virology* 1994; **200**: 436-446
31 **Pereira LM**, Melo MC, Saleh MG, Massarolo P, Koskinas J, Domingues AL, Spinelli V, Mies S, Williams R, McFarlane IG. Hepatitis C virus infection in Schistosomiasis mansoni in Brazil. *J Med Virol* 1995; **45**: 423-428
32 **Ferrante A**, Kiroff GK, Drew PA. Elevated natural killer (NK) cytotoxicity of mononuclear leucocytes from splenectomized patients: increase in Leu-7+ and Leu-11+ leucocytes. *Clin Exp Immunol* 1986; **64**: 173-180
33 **Abe T**, Masuda T, Satodate R. Phagocytic activity of Kupffer cells in splenectomized rats. *Virchows Arch A Pathol Anat Histopathol* 1988; **413**: 457-462
34 **Tsukiyama-Kohara K**, Kohara M, Yamaguchi K, Maki N, Toyoshima A, Miki K, Tanaka S, Hattori N, Nomoto A. A second group of hepatitis C viruses. *Virus Genes* 1991; **5**: 243-254
35 **Simmonds P,** McOmish F, Yap PL, Chan SW, Lin CK, Dusheiko G, Saeed AA, Holmes EC. Sequence variability in the 5' non-coding region of hepatitis C virus: identification of a new virus type and restrictions on sequence diversity. *J Gen Virol* 1993; **74 (Pt 4)**: 661-668
36 **Young KK,** Archer JJ, Yokosuka O, Omata M, Resnick RM. Detection of hepatitis C virus RNA by a combined reverse transcription PCR assay: comparison with nested amplification and antibody testing. *J Clin Microbiol* 1995; **33**: 654-657
37 **Nolte FS,** Thurmond C, Fried MW. Preclinical evaluation of AMPLICOR hepatitis C virus test for detection of hepatitis C virus RNA. *J Clin Microbiol* 1995; **33**: 1775-1778
38 **Oshimi K**, Oshimi Y, Satake M, Mizoguchi H. Natural killer-mediated lysis of normal and malignant target cells, and its regulation by monocytes. *J Exp Med* 1985; **162**: 472-486
39 **Ikuta S,** Ono S, Kinoshita M, Seki S, Hiraide H, Mochizuki

H. Enhanced interferon-gamma production and bacterial clearance in the liver of splenectomized mice in the models of Escherichia coli injection or intestinal obstruction. *Shock* 2004; **21**: 452-457

40 **Podevin P,** Calmus Y, Bonnefis MT, Veyrunes C, Chereau C, Poupon R. Effect of cholestasis and bile acids on interferon-induced 2',5'-adenylate synthetase and NK cell activities. *Gastroenterology* 1995; **108**: 1192-1198

41 **Kato N**, Yokosuka O, Hosoda K, Ito Y, Ohto M, Omata M. Quantification of hepatitis C virus by competitive reverse transcription-polymerase chain reaction: increase of the virus in advanced liver disease. *Hepatology* 1993; **18**: 16-20

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