Title: Influence of splenectomy in patients with liver cirrhosis and hypersplenism

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Short title: Splenectomy for cirrhosis

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Abstract

Aim Splenectomy improves hypersplenic thrombocytopenia in cirrhotic patietnts with hypersplenism. However, the long term influence of splenectomy has not been calrified. We examined whether splenectomy improved liver fibrosis and casused immunological changes.

Material and Methods We collected liver and spleen specimens and peripheral blood (PB) from 26 patients with hepatitis C virus (HCV)-related liver cirrhosis. An immunohistochemical examination of CD4, CD8, forkhead box P3 (FOXP3), granzyme B, and transforming growth factor (TGF)-β1, and Masson's Trichrome stain were performed in spleen and liver tissues and in 7 cases of follow-up liver biopsy sections obtained after splenectomy. We obtained PB before and at various intervals after splenectomy. We also examined the ratio of $CD4^+$ and $CD8^+$ lymphocytes in PB using flow cytometry. **Results** We observed improvements in liver fibrosis in 4 biopsy specimens obtained after splenectomy, in which fibrotic areas significantly decreased from 19.5% to 8.2% ($p<0.05$). Increases were also observed in the ratio of $CD8^+$ cells in PB after splenectomy, which resulted in a significant decrease in the $CD4^+/CD8^+$ ratio ($p<0.001$). The carcinogenic rate in patients with a $CD4^+/CD8^+$ ratio that decreased by more than 0.5 one month after splenectomy was significantly lower than that in patients with a ratio that decreased by less than 0.5 ($p<0.05$).

Conclusions Splenectomy may improve liver fibrosis and cause beneficial immunological changes in cirrhotic patients with hepatitis. Improvements in anti-tumor mechanisms can be

also expected.

Key words: Splenectomy, Liver fibrosis, Liver cirrhosis, $CD4^+$ CTLs, $CD8^+$ CTLs

Introduction

Splenectomy is a common treatment used to improve hypersplenic thrombocytopenia in cirrhotic patients with splenomegaly in Japan [1-7]. Splenectomy has recently been applied as another option to cure hepatocellular carcinoma (HCC) and for cirrhotic patients with no

potential donor for liver transplantation. Thus, the clinical application of splenectomy has been expanded; however, the immunophysiology of the spleen in cirrhotic patients and the long term outcome after splenectomy have not been clarified [8-14]. This study was designed to clarify the long term changes and prediction of HCC development following splenectomy, with a focus on hepatic fibrosis and immunology. Regarding hepatic fibrosis, Akahoshi et al reported that TGF-β1 derived from the spleen could have an inhibitory role in healing iver cirrhosis by inhibiting the regeneration of the damaged liver[15] and we experimentally confirmed that splenectomy significantly reduced liver fibrosis and decreased TGF-β1 in the serum of a dimethylnitrosamine-induced cirrhotic rat model [16]. However, no studies have yet described a reduction in hepatic fibrosis following splenectomy in humans.

The spleen plays an important role in the immune response; however, the functional aspects of the spleen in cirrhotic patients with HCV infection are largely unknown [2, 17]. Hashimoto et al reported that splenectomy was followed by an increased ratio of interferon (IFN)-γ to interleukin (IL)-10 and a reduction in programmed death (PD)-1-expressing $CD4^+$ T cells in PB [7]. In order to clarify chronological changes in immunity after splenectomy, we examined liver and spleen tissues and sera to assess $CD4^+$ and $CD8^+$ cytotoxic T lymphocytes (CTLs), and regulatory T (Treg) cells [18, 19]. TGF-β1 was also examined as it is a multifunctional cytokine that inhibits the growth of tumor cells [20-23] and liver regeneration by facilitating tissue fibrosis in the liver [16].

Host immunoreactions against cancer were shown to be closely related to cellular immunity by $CD8^+$ CTLs and Treg cells, produced by T lymphocytes, and $CD8^+$ CTLs in particular [19]. The level of Treg cells, characterized by the expression of forkhead box P3 (FOXP3) transcription factor in the PB and tumor tissues of patients with HCC, was elevated and appeared to be negatively correlated with prognosis [21, 24, 25].

In the present study, we examined whether splenectomy could improve liver fibrosis, cause immunological changes, especially in CTLs, or be used to predict the risk of carcinogenesis.

Materials and methods

1. Patients and samples (Table 1)

At the Surgery Department of Kurume University Hospital, 26 patients (Child A; 16 cases, Child B, C; 10 cases) with HCV-related liver cirrhosis (with HCC; 7 cases, without HCC; 19

cases) and hypersplenism underwent splenectomy (splenectomy group). The purpose of splenectomy was to improve hypersplenic thrombocytopenia and introduce interferon for clearance of the HCV virus. Forty-eight patients who underwent hepatectomy due to liver tumors were recruited as controls (control group 1). PB samples from 10 healthy adult volunteers (control group 2) and spleen tissues obtained by splenectomy from 7 patients because of trauma (control group 3) were also used as controls. In addition, all patients were HIV-negative. Patients received no medical treatment except splenectomy during the study period. All samples were studied after obtaining the appropriate institutional informed consent. We also obtained permission from the Ethical Review Board.

Liver tissue

A total of 26 pieces from the resected liver specimens of patients with HCV-related liver cirrhosis and hypersplenism who underwent splenectomy were also examined for the immunohistochemical expression of $CD4^+$ lymphocytes, $CD8^+$ lymphocytes, FOXP3, granzyme B, and TGF-β1 positive cells (Fig. 1). We classified liver specimens into 5 stages according to the degree of fibrosis as follows: F0, no fibrosis in the portal tract; F1, portal fibrosis without septa; F2, portal fibrosis with a few septa; F3, numerous septa without cirrhosis; and F4, cirrhosis. We collected resected liver specimens from 10 cases each of F1, F2, F3, and F4 with HCV-related liver disease. We also collected specimens from 8 cases of liver hemangioma of F0 with both negative HBs-Ag and HCV-Ab. Follow-up liver biopsy

sections were obtained from the same part of the liver if possible from 7 of the 26 patients at various intervals after splenectomy (Table 2). These sections were used for CD4 and CD8 immunostaining and Masson's Trichrome staining for the morphometric evaluation of fibrotic areas.

Spleen tissue

A total of 26 spleens with HCV-related liver cirrhosis and hypersplenism were examined for the immunohistochemical expression of CD4 positive lymphocytes, CD8 positive lymphocytes, FOXP3, granzyme B, and TGF-β1 positive cells. We measured the same parameters in spleens from the 7 control cases in control group 3 as a non-cirrhotic control (Fig. 1). Spleen and liver tissues were pathologically assessed by two pathologists (Y.N and M.K).

Peripheral blood cells

PB samples were serially collected from 26 patients with HCV-related liver cirrhosis and hypersplenism just before and 14 days, 1 month, 3 months, 6 months, and 1 year after splenectomy, respectively. We examined the ratio of $CD4^+$ T cells to all lymphocytes, $CD8^+$ T cells to all lymphocytes, and the $CD4^+/CD8^+$ ratio in PB samples using flow cytometry. And TGF-β1 levels in PB were also measured using enzyme-linked immunoassays in the sera just before and 14 days, 1 month, 3 months, 6 months, and 1 year after splenectomy, respectively. Patients were excluded from the protocol if IFN or other therapeutics were introduced for the

liver disease. Ten healthy adult volunteers in control group 2 without a history of liver disease or splenomegaly were also recruited as controls, and samples were collected only one time.

2. Immunohistochemical analysis

All fresh specimens were fixed by 10% formalin, and paraffin-embedded tissue samples were cut at a thickness of 4 μm, examined on a coated slide glass, and labeled with the following antibodies using the Bond-Max autostainer (Leica Microsystems, Newcastle, UK) and DAKO autostainer (DakoCytomation, Glostrup, Denmark): CD4 (×200, Leica Microsystems, Newcastle, UK): CD8 (×200, Leica Microsystems, Newcastle, UK), Granzyme B (×50, Leica Microsystems, Newcastle, UK), TGF-β1 (×300, Santa Cruz Biotechnology, Heidelberg, Germany), and Foxp3 (×600, Abcam Cambridge, USA).

Immunohistochemical examinations with CD4, CD8, granzyme B, and TGF-β1 were performed on the same fully automated Bond-Max system using onboard heat-induced antigen retrieval with ER2 for 10 min and the Refine polymer detection system (Leica Microsystems, Newcastle, UK). DAB was used as the chromogen for all immunostaining. Foxp3 immunostaining was carried out using the DAKO autostainer with the ChemMate ENVISION method (DakoCytomation, Glostrup, Denmark). Briefly, specimens were boiled in a microwave for 30 min in 1 mmol/L EDTA, pH 9.0, and target retrieval solution (DakoCytomation, Glostrup, Denmark) to recover antigens, and the specimens were then

incubated with the antibody at 4°C overnight. After washing in tris-buffered saline (TBS), slides were incubated with the labeled polymer-HRP secondary antibody for 30 minutes at room temperature. After washing in TBS, slides were visualized using DAB.

3. Detection of immune function using flow cytometry

T lymphocyte subsets in PB such as CD4, CD8, and CD4/8 were determined by flow cytometry, and the monoclonal antibodies of CD4 and CD8 (labeled CD4-FITC, CD-8-RD1) were purchased from Beckman Coulter, USA.

4. Result assessment

Assessment criteria for lymphocytes and other positive cells counts: the number of lymphocytes and other positive cells were counted in 20 areas within a specimen under high power fields (x40 objective, x10 eyepiece). Ten areas of white and red pulp were assessed in the spleen, respectively, and 10 periportal areas and 10 hepatic lobule areas (Fig. 1) were assessed in a non-tumor area of the liver.

Morphometric analysis (computer-image analysis) was performed in the following manner on specimens stained with Masson’s Trichrome. The equipment used to assess morphometry consisted of a light microscope, a three-color charge-coupled device camera, and a high resolution computer image-analysis system (WinRooF software package: version 6.1, Mitani

corporation, Fukui, Japan). The magnified images (40×) of specimens captured by the camera mounted on the microscope were sent to the image-analyzing computer. Collagen fibers stained with Masson's Trichrome were then selected. In this study, this scanning procedure was repeated 10 times in random areas. The area of fibrosis (AF) was defined as the ratio (%) of the whole area of collagen fibers to that of the liver tissue scanned.

5. Statistical analyses

Statistical analysis was performed using the Student's t test. A p-value of less than 0.05 was considered to be significant.

The follow-up time was calculated as the interval between the date of surgery and intervention of the medical treatment, last follow-up, or recognition of HCC. Survival rates or failure rates were analyzed with the Kaplan-Meier method using the log-rank test to assess differences between curves. A p-value of less than 0.05 was considered to be significant. Statistical calculations were performed using the JMP software package (release 10, SAS Institute, Cary, NC, USA).

Results

1. Liver

In the seven follow-up liver biopsy sections (Table 2) available for histological examination, liver fibrosis in the hepatic lobules improved from F4 to F3 in 4 cases (Cases 4-7: average,

268.5±168.6 days; range, 42-431 days) (Fig. 2-a). Improvements were not observed in the remaining 3 cases (Cases 1-3: average, 312±279.1 days; range, 24-581 days) (Fig. 2-b). There were no statistical differences in the duration between the improvement cases and non-improvement cases ($p=0.80$). Conducting an evaluation was difficult because only a few specimens were available; however, no significant differences in clinical profiles were observed among the 7 patients. In 4 of these cases (cases 4-7), the ratio significantly decreased from 19.5% to 8.2% ($p<0.05$) (Fig. 2-b), while the average AF in the remaining 3 cases (cases 1-3) increased from 8.0% to 13.1% ($p=0.15$). The 4 cases of improved fibrosis were all Child-Pugh A, and one of the 3 cases that showed no improvement was Child-Pugh B. In addition, AF before splenectomy was slightly higher in the improvement cases than in the non-improvement cases, while the $CD4^+/CD8^+$ ratio before splenectomy was lower in the improvement cases than in the non-improvement cases ($p<0.05$). Histopathologically, $CD4^+$ and $CD8^+$ lymphocytes were mainly seen in the periportal area, and $CD4^+$ lymphocytes were rarely seen in the hepatic lobules. The epithelial cells, fibroblasts, monocytes, and macrophages also produced TGF-β1 [4, 21, 26]. However, we picked up and counted the TGF-β1 positive cells that were seen in the lymphocytes and found that these cells were distributed diffusely in the hepatic lobules and periportal area. The distribution pattern of Treg and granzyme B was the same as that of $CD4^+$ and $CD8^+$ lymphocytes, respectively. No significant differences were observed in the $CD4^+/CD8^+$ ratio ($p=0.21$) in liver specimens,

regardless of the association of HCC. The $CD4^+/CD8^+$ ratio ($p<0.05$) and $Foxp3/CD4^+$ ratio ($p<0.001$) significantly increased with the progression of liver fibrosis (from F0 to F4). However, the granzyme B/ $CD8^+$ ratio was approximately constant, and was unrelated to the progression of liver fibrosis ($p=0.32$).

The number of TGF-β1 positive cells in livers with HCC was slightly higher than that in livers without ($p=0.06$), and the number of TGF-β1 positive cells also significantly increased with the progression of liver fibrosis ($p<0.001$) (Fig. 3).

2. Spleen

Histopathologically, $CD4^+$ and $CD8^+$ lymphocytes were found more in the white pulp than in the red pulp. The results of the clinicopathological analysis showed that the $CD4^+/CD8^+$ ratio in spleens with HCV-related liver cirrhosis and hypersplenism was higher than that in the spleens of control group 3 ($p=0.06$). The $Foxp3/ CD4^+$ ratio in control group 3 was higher than that in cases of hypersplenism ($p<0.05$), and no significant differences in the granzyme B/ $CD8^+$ ratio ($p=0.82$)were observed between the splenectomy group and control group 3 (data not shown).

3. Peripheral blood

The ratio of $CD4^+$ T cells to all lymphocytes and the $CD4^+/CD8^+$ ratio in PB samples obtained from 26 patients before splenectomy were significantly higher than those from control group 2 ($p<0.01$, $p<0.05$). In contrast, the ratio of $CD4^+$ T cells to all lymphocytes

significantly decreased 1 year after splenectomy ($p<0.001$), while the ratio of $CD8^+$ T cells to all lymphocytes slightly increased ($p=0.07$), resulting in a significant decrease in the $CD4^+/CD8^+$ ratio ($p<0.001$) (Fig. 4).

TGF-β levels were higher in PB samples from patients with HCC than in those without. TGF-β1 levels slightly increased in PB samples 1 month after splenectomy, then decreased, and subsequently returned to the level measured before splenectomy in one year.

4. Relationship of the $CD4^+/CD8^+$ ratio between PB and the spleen or liver

In the splenectomy group, the $CD4^+/CD8^+$ ratio in PB had a significant positive correlation with the $CD4^+/CD8^+$ ratio in the spleen ($p<0.05$), and was also positively associated with the liver ($p=0.07$). As a result, a significant positive correlation was observed between the $CD4^+/CD8^+$ ratio in the spleen and that in the liver ($p<0.05$) (Fig. 5).

5. Correlation between the $CD4^+/CD8^+$ ratio and clinical prognosis

We compared the $CD4^+/CD8^+$ ratio between PB obtained pre-splenectomy and 1 month after splenectomy ($n=19$). The median of differences between pre-splenectomy and 1 month after splenectomy was 0.5. The occurrence of HCC was significantly lower in cases in which the difference in the $CD4^+/CD8^+$ ratio between the perioperative period and 1 month later was over 0.5 (>0.5 vs. <0.5, $p<0.05$) (Fig. 6A).

A positive correlation in PB was observed between the $CD4^+/CD8^+$ ratio before splenectomy and differences in the $CD4^+/CD8^+$ ratio between pre-splenectomy and 1 month after

splenectomy ($p<0.001$). As the median of the preoperative $CD4^+/CD8^+$ ratio was 1.7, the postoperative (1 month after splenectomy) $CD4^+/CD8^+$ ratio significantly decreased in groups in which the preoperative value was larger than 1.7 (Fig. 6B, C).

Discussion

Previous studies have shown that splenectomy was effective in improving pancytopenia, the decompression of portal hyperpressure, and liver function [1, 2, 27, 28]. Morinaga et al reported that splenectomy significantly improved liver fibrosis with a reduction in plasma TGF-β1 levels in the rat. However, all these reports of hepatic fibrosis were conducted in animal models [1, 16, 29, 30] whereas the present study described improvements in liver fibrosis after splenectomy in humans. Interestingly, the $CD4^+/CD8^+$ ratio changed after splenectomy without other treatment. However, many confounding factors may be implicated in this change. It is likely that patients with a high fibrotic area in their liver specimens had a high $CD4^+/CD8^+$ ratio; therefore, we may expect a decrease in the $CD4^+/CD8^+$ ratio after splenectomy. A decrease in Treg cells that stimulate TGF-β1 may lead to alleviation of fibrosis.

Because the immune function of $CD4^+$ CTLs, $CD8^+$ CTLs, and the $CD4^+/CD8^+$ ratio is affected by a wide variety of factors including recent exercise, poor nutrition, and coincident acute viral infections, it is difficult to evaluate immune function using only $CD4^+$ CTLs, $CD8^+$ CTLs, and the $CD4^+/CD8^+$ ratio. However, in our study, the ratio of $CD4^+$ T cells to all lymphocytes in PB was significantly decreased in cirrhotic patients after splenectomy, while the ratio of $CD8^+$ T cells to all lymphocytes slightly increased, resulting in a significant decrease in the $CD4^+$ /$CD8^+$ ratio. The $CD4^+/CD8^+$ ratios in PB, spleens, and livers were

significantly higher in patients with hypersplenism and in those in whom liver fibrosis had progressed than in the controls. As a positive correlation was observed between the $CD4^+/CD8^+$ ratios in the spleens, livers, and PB, it is possible to expect to predict the immunological state of the liver and spleen from the immunological state of PB. In addition, carcinogenesis was significantly lower in groups in which a large difference in the $CD4^+/CD8^+$ ratio was observed between before and after splenectomy or in those with a high $CD4^+/CD8^+$ ratio before splenectomy though there were few cases that we could observed. The $CD4^+$ /$CD8^+$ ratio is likely to be a key parameter for appropriate TIL function, and was shown to be different in different types of cancer [2, 31-35]. Host immune responses to cancer were reported to depend on T lymphocytes, particularly $CD8^+$ lymphocytes [18, 19, 24, 36-39]. An increase in their ratio after splenectomy and the consequent decrease in the $CD4^+/CD8^+$ ratio observed in this study may be a positive change in terms of immunology against HCC. Such a change was particularly marked in patients with a high $CD4^+/CD8^+$ ratio before splenectomy.

In our study, the $CD4^+$ /$CD8^+$ ratio also significantly increased as the fibrosis of non-tumor areas in the liver tissue progressed. These significant differences were observed regardless of the HCC status. Although the cause of these differences is unknown, it appears to depend on the background of histological factors in the liver such as fibrosis. Many studies have investigated the relationship between tumors, Treg, and TGF-β [20-22, 25, 40]. Guo-He et al

showed that the expression of TGF-β appeared to be positively correlated with Treg in HCC tissue. The 5-year survival rate was significantly lower in patients with HCC tissues with high Treg cell infiltration than in those with low infiltration [20, 22, 36, 41]. Our study also revealed that Treg cells were positively correlated with TGF-β1 positive cells even in "non-tumor areas" of liver tissue, and that TGF-β1 positive cells were positively correlated with liver fibrosis. There were no significant differences of TGF-β1 before and after splenectomy. The reason for the chronological changes in TGF-β1 levels after splenectomy is unknown because various factors including platelets may be involved in the production of TGF-β1. We also found a slightly higher number of TGF-β1 positive cells in non-tumor areas in the liver tissue of patients with HCC than in those without. Furthermore, the number of TGF-β1 positive cells significantly increased with the progression of liver fibrosis [4, 21, 26, 42].

In conclusion, splenectomy in cirrhotic patients with hepatitis may be able to improve liver fibrosis, may cause beneficial immunological changes, and may lower the risk of carcinogenesis. It seems necessary to accumulate furthermore cases for convinced conclusion.

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Figure legends

Fig. 1 Immunohistochemical staining of spleen and liver specimens with FOXP3, CD4, CD8, granzyme B, and TGF-β1 in the spleen and liver.

Fig. 2-a Improvements in liver fibrosis.

Distortions in hepatic lobules improved in the liver biopsy sections of 4 cases after splenectomy, and fibrotic areas significantly decreased from 19.5% to 8.2% in these sections.

Fig. 2-b Changes in the fibrotic areas of 7 patients at various intervals.

●—● shows patients in whom the fibrotic area significantly decreased after splenectomy.

▲···▲ shows patients in whom fibrosis deteriorated.

Fig. 3 Correlation between TGF-β1 positive cells and fibrosis in the liver.

The number of TGF-β1 positive cells also significantly increased with the progression of Liver fibrosis

Fig. 4 Changes in peripheral blood after splenectomy.

(pre, preoperative; d, days; M, months; y, year)

The ratio of $CD4^+$ T cells to all lymphocytes significantly decreased 1 year after splenectomy, while the ratio of $CD8^+$ T cells to all lymphocytes slightly increased, resulting in a significant decrease in the $CD4^+/CD8^+$ ratio.

Fig. 5 Correlations between the $CD4^+/CD8^+$ ratios in the spleen, liver, and PB.

A significant positive correlation was observed between the $CD4^+/CD8^+$ ratio in the spleen and that in the liver.

Fig. 6A Correlation between carcinogenesis, the perioperative period, and 1 month later.

The occurrence of HCC was significantly lower in cases in which the difference in the $CD4^+/CD8^+$ ratio between the perioperative period and 1 month later was over 0.5.

Fig. 6 B, C Correlation in PB between the $CD4^+/CD8^+$ ratio before surgery and differences in the $CD4^+/CD8^+$ ratios before splenectomy and 1 month after splenectomy.

B) A positive correlation in PB was observed between the $CD4^+/CD8^+$ ratio before splenectomy and differences in the $CD4^+/CD8^+$ ratio between pre-splenectomy and 1 month after splenectomy.

C) The postoperative (1 month after splenectomy) $CD4^+/CD8^+$ ratio significantly decreased in groups in which the preoperative value was larger than 1.7

Table 1 Subject characteristics

Variables	Results
Splenectomy group: Splenectomy (26 cases; 7 with HCC, 19 without HCC)	
Age median (range)	60.4±1.36 (46-75)
Gender (male/female)	12 / 14
Virus infection [HCV(+)]	26
Fibrosis (F0/F1/F2/F3/F4)	0/0/0/0/26
Child-Pugh classification (A/B/C)	16/8/2
Tumor nodules (presence/absence)	7/19
Weight of the spleen (g)	510.4±55.6 (125-1065)
Control 1: Hepatectomy with HCC (48 cases)	
Age median (range)	70.5±1.33 (42-82)
Gender (male/female)	29/19
Virus infection [HCV(+)]	40
Fibrosis (F0/F1/F2/F3/F4)	8/10/10/10/10
Tumor nodules (presence/absence)	48/0
Control 2: Healthy adult volunteers (10 cases)	
Age median (range)	40.1±2.97 (32-57)
Gender (male/female)	3/7
Control 3: Splenectomy control (7 cases; trauma)	
Age median (range)	59.8±6.27 (36-82)
Gender (male/female)	6 / 1

Continuous variables are expressed as the mean ±standard deviation (SD)
HCV: hepatitis C virus; fibrosis: F0, no fibrosis in the portal tract;
F1, portal fibrosis without septa; F2, portal fibrosis with few septa;
F3, numerous septa without cirrhosis; and F4, cirrhosis

Table 2
Clinical and pathological findings of 7 patients who underwent follow-up liver biopsies

Case	Age	Gender	Activity	Child-Pugh(score)	CD4/8	Follow-up range(days)	Before (%)	After (%)	Rate of change
1	63	M	1	A(5)	1.73	581	6.59	18.31	2.78
2	58	M	2	A(5)	1.22	24	7.38	8.99	1.22
3	58	M	2	B(7)	1.57	333	9.92	12.02	1.21
4	52	M	2	A(5)	1.08	431	16.71	5.10	0.30
5	74	M	2	A(6)	0.63	353	20.02	6.31	0.32
6	53	F	2	A(6)	0.93	248	30.03	13.34	0.44
7	59	M	2	A(5)	0.95	42	11.27	8.05	0.71

Activity: A0, none; A1, portal inflammation only; A2, mild interface hepatitis; A3, moderate interface hepatitis; A4, severe interface hepatitis
Before: the rate of fibrotic areas before splenectomy, after; the rate of fibrotic areas after splenectomy

Fig. 1

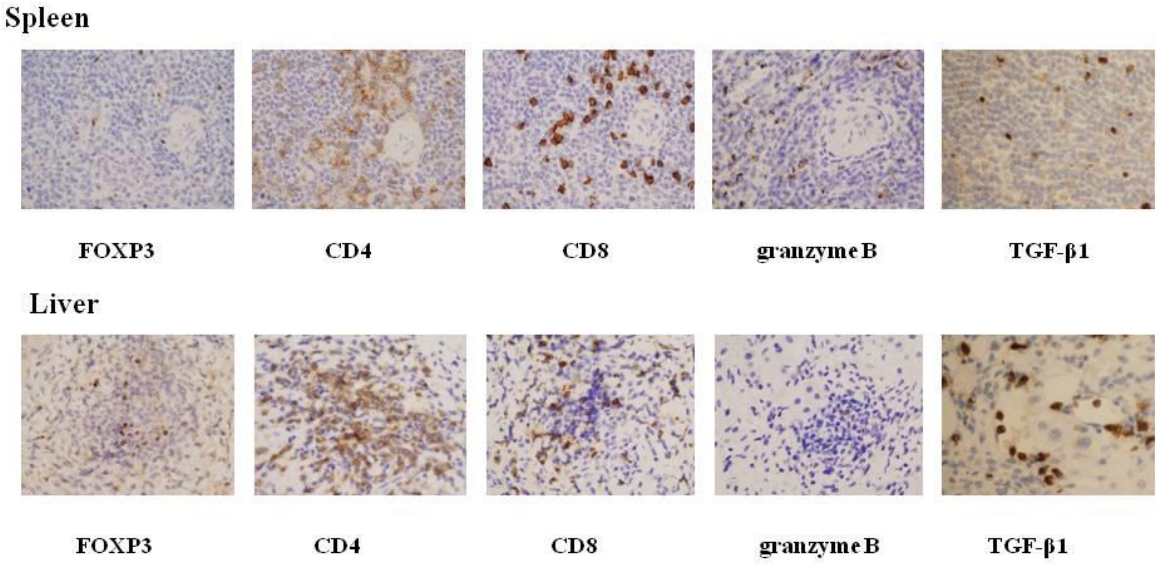
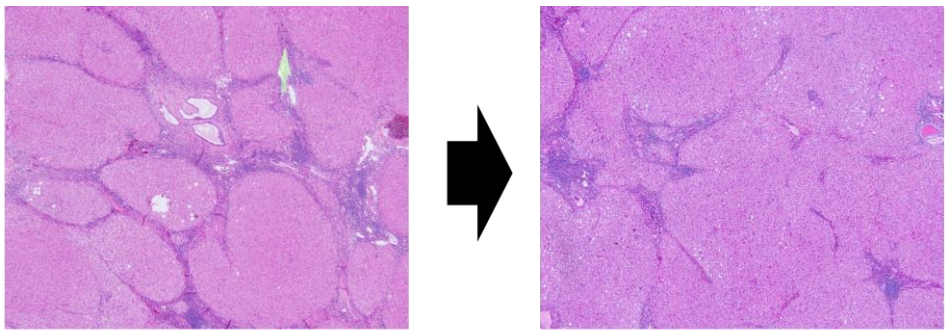

Spleen
FOXP3
CD4
CD8
granzyme B
TGF-β1
Liver
FOXP3
CD4
CD8
granzyme B
TGF-β1

Fig.2-a



At the time of the surgery

1 year after the splenectomy

Fig. 2-b

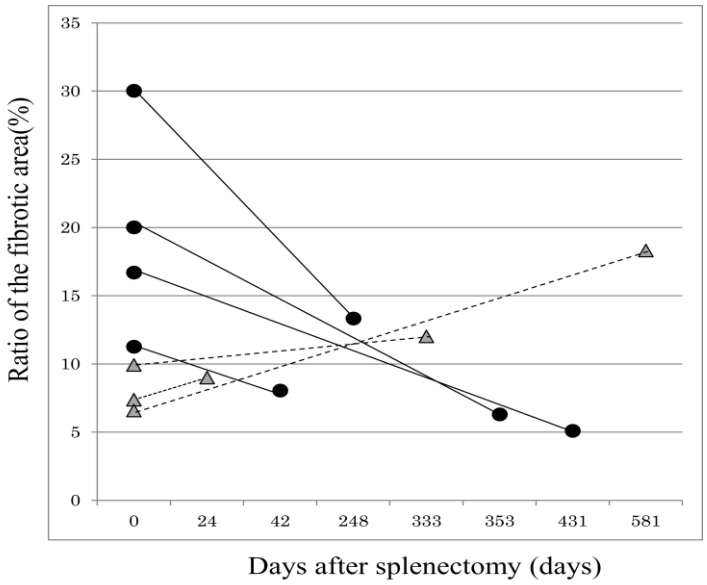

35
30
25
20
15
10
5
0
Ratio of the fibrotic area(%)
0
24
42
248
333
353
431
581
Days after splenectomy (days)

Fig.3

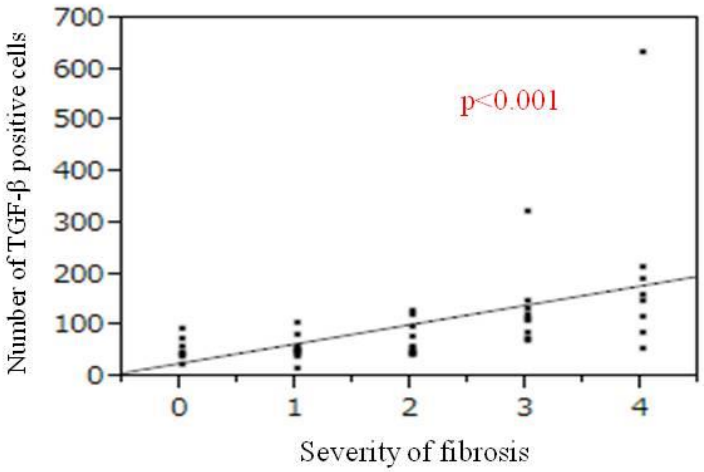

Number of TGF-β positive cells
700
600
500
400
300
200
100
0
p<0.001
0
1
2
3
4
Severity of fibrosis

Fig. 4

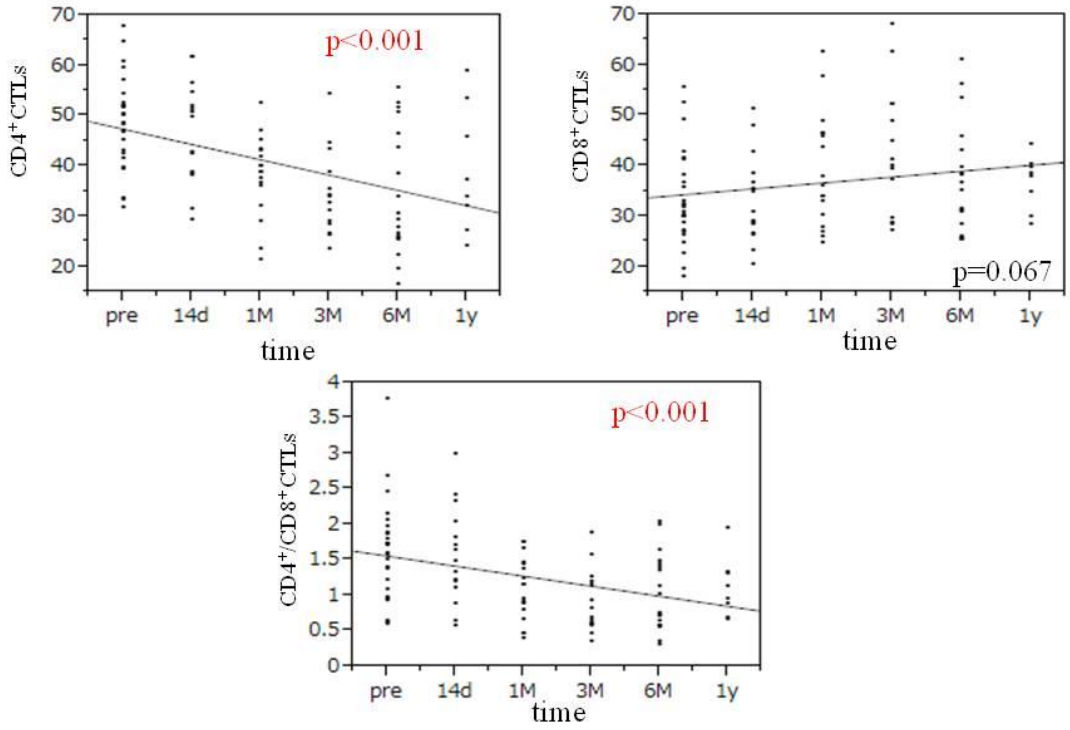

CD4+CTLs
p<0.001
pre
14d
1M
3M
6M
1y
time
CD8+CTLs
p=0.067
CD4+/CD8+CTLs
p<0.001

Fig. 5

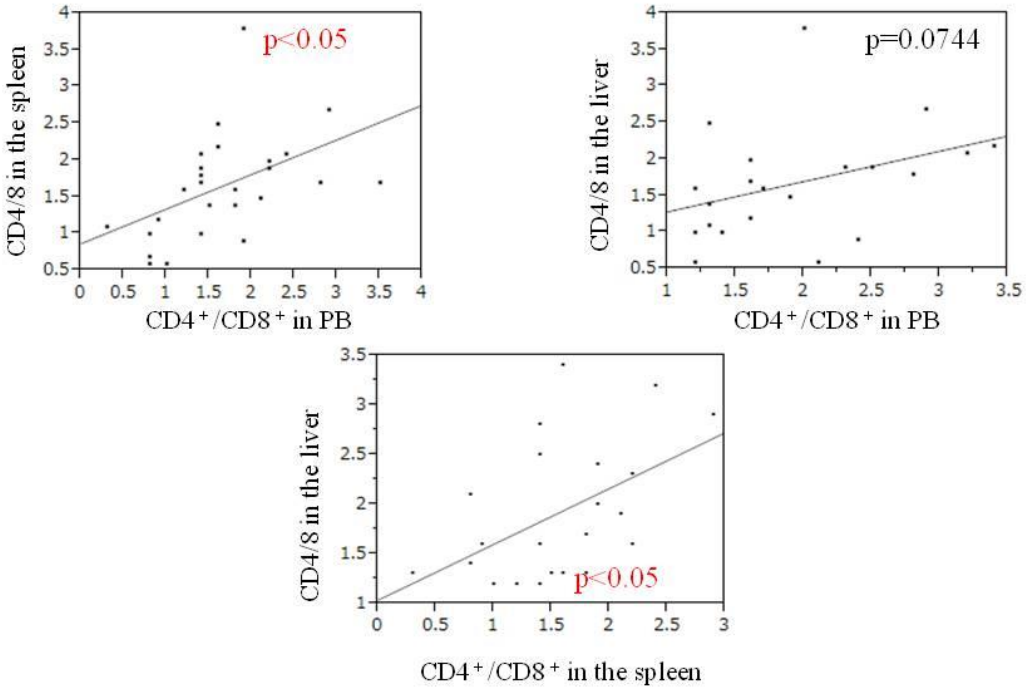

p<0.05
CD4/8 in the spleen
CD4+/CD8+ in PB
p=0.0744
CD4/8 in the liver
CD4+/CD8+ in PB
p<0.05
CD4/8 in the liver
CD4+/CD8+ in the spleen

Fig.6A

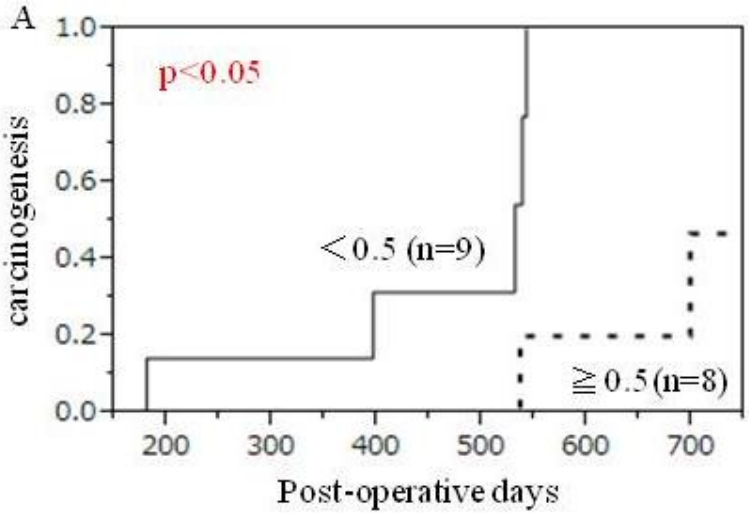
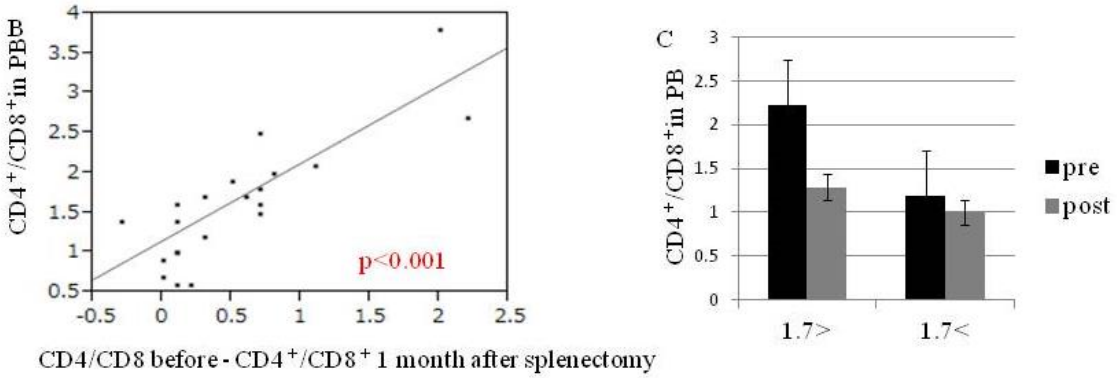

A
1.0
0.8
0.6
0.4
0.2
0.0
carcinogenesis
p<0.05
＜0.5 (n=9)
≧0.5 (n=8)
200
300
400
500
600
700
Post-operative days

Fig. 6B, C


B
CD4+/CD8+in PB
p<0.001
CD4/CD8 before - CD4+/CD8+ 1 month after splenectomy
C
CD4+/CD8+in PB
1.7>
1.7<
pre
post