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Suppressive Effects of Interleukin-18 on Liver Function in Rat Liver Allografts

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Background. **Interleukin-18 (IL-18) is a potent proinflammatory cytokine that augments both innate and acquired immune responses. It is also a crucial regulator of lymphocyte production of interferon-γ (IFN-γ), which can promote acute cellular rejection of transplanted solid organs.**

Methods. **To evaluate the role of IL-18 in liver transplantation, we constructed an adenoviral vector encoding IL-18 binding protein (Adex-IL18bp), which specifically suppressed the biologic activity of IL-18, and examined the effect of this suppression on liver allografts by using a high-responder rat model (ACI to Lewis) of orthotopic liver transplantation (OLTx). Donor rats were given one intravenous injection of Adex-IL18bp or Adex-LacZ (control vector) 2 d before OLTx.**

Results. **Seven days after OLTx, overexpression of IL-18bp resulting from the adenovirus gene transfer was associated with significantly decreased serum alanine aminotransferase levels and less histologic hepatic injury in recipient rats with Adex-IL18bp-pretreated donors compared with Adex-LacZ controls. Adex-IL18bp pretreatment also significantly prolonged rat/allograft survival, inhibited expression of IFN-γ, and reduced levels (*versus* control values) of both CXCL10 and CX3CL1, which can be induced by IFN-γ.**

Conclusion. **These results suggest that IL-18 has an important role in liver allograft rejection through IFN-γ and chemokines and that specific suppression of IL-18 may improve liver function early after transplantation.** © 2012 Elsevier Inc. All rights reserved.

Key Words: **adenovirus vector; interferon-γ; interleukin-18; liver allograft.**

INTRODUCTION

Much progress has been made in identifying the cellular and molecular mechanisms by which cytokines participate in rejection processes after organ transplantation. Although Th1- and Th2-type cytokine profiles have been associated with, respectively, rejection and tolerance, recent studies have suggested that the mechanism of allograft rejection involves not simply Th1/Th2 deviations, but that regulation within the cytokine network is substantially more complicated, with substantial contributions from several cytokines. One cytokine that may affect both Th1 and Th2 responses is interleukin (IL)-18 [1].

IL-18 is produced not only by various immune cells but also by non-immune cells such as intestinal and airway epithelial cells. In collaboration with IL-12, IL-18 stimulates Th1-mediated immune responses, which play a critical role in host defense against intracellular microbes through the induction of interferon-γ (IFN-γ).

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However, overproduction of IL-12 and IL-18 induces severe inflammatory disorders, suggesting that IL-18 is a potent proinflammatory cytokine that is pathophysiologically involved in several inflammatory conditions [2]. Moreover, IL-18 has been found to be significantly up-regulated in both the serum and the alloresponse site in patients with acute rejection of a kidney allograft [1]; therefore, this cytokine may play an important part in acute rejection of transplanted solid organs.

IL-18 binding protein (IL-18bp) was first identified in the urine of healthy persons and purified by using IL-18-coupled beads [3]. IL-18bp is thought to be a soluble decoy receptor because it can specifically block binding of mature IL-18 to its authentic receptor and thereby inhibit IL-18-induced IFN-γ production [2]. Since IL-18 is an early stimulant of Th-1 cells, IL-18bp probably has an important role in regulation of the immune response [2].

Acute rejection of liver allografts is characterized histologically by a mixed portal-tract infiltrate containing mononuclear cells. An accumulation of activated lymphocytes in the allograft is essential to the pathogenesis of tissue injury. The mechanism by which these lymphocytes are recruited to the graft from the circulation is poorly understood, but it probably involves local chemotactic factors that promote migration, positioning, and retention of effector cells in the graft [4, 5]. Chemokines are expressed and secreted by a wide variety of cell types, including lymphocytes [6] and endothelial components of allografts activated during rejection [4, 7-9]. Several studies have shown that both CXCL10 (IFN-γ-inducible protein 10) and CX3CL1 (fractalkine), which are induced by IFN-γ [10], are up-regulated during rejection of murine cardiac [11, 12] and rat liver allografts [5].

The aim of the current study was to assess the role of IL-18 in liver transplantation. We constructed an adenoviral vector encoding IL-18bp, which specifically suppressed the biologic activity of IL-18. We then examined the effects of this inhibition in a high-responder model of rat orthotopic liver transplantation (OLTx).

MATERIALS AND METHODS

Cloning of Rat IL-18bp cDNA and Construction of Adenoviral Vector

Rat IL-18bp cDNA was amplified from the Rat Liver Marathon-Ready cDNA library (Clontech, Tokyo, Japan) by using polymerase chain reaction (PCR) with the following primers: 5′-CATGCAGGTCTCCCATGAGACACTGTGGCTGTGCAGCAG-3′ and 5′-TGGTTGCTGGAGTGGGGCCCCTGGGCCTGCTGATCTGG-3′ (underlining indicates the restriction-enzyme sites of *Bsa*I and *Xho*I, respectively). A plasmid vector, pTriEx-rIL18bpHis, which expresses rat IL-18bp with a C-terminal 6xHis tag, was constructed by inserting the amplified fragment digested with *Bsa*I and *Xho*I into the *Nco*I-*Xho*I site of the plasmid pTriEx1.1 (Novagen, Tokyo). The sequence of the insert was then confirmed.

The replication-defective adenoviral vector containing the CAG promoter, *E coli lac*Z gene, and poly(A) sequence (that is, the Adex-LacZ control) was kindly provided by Dr. I. Saito, Institute of Medical Science, University of Tokyo [13]. Another replication-defective adenoviral vector (Adex-IL18bp), which expresses the rat IL-18bp gene derived from pTriEx-rIL18bpHis, was constructed by using an adenovirus expression vector kit (Takara Bio, Tokyo). The amplified fragment containing the coding region with the primers 5′-CATTGAATTCACAATCAAAGGAGATATACC-3′ and 5′-TGATAGAATTCCTGCACCTGAGGTTAATCAC-3′ from pTriEx-rIL18bp was inserted into the unique *Swa*I site of the adenovirus genome in the cassette cosmid pAxCAwt. After sequencing of the coding region, the cosmid bearing an expression unit was co-transfected into human embryonic kidney 293 cells, together with the adenovirus DNA-terminal protein complex. The cloned recombinant adenoviruses were purified by using cesium chloride ultracentrifugation. Titers were assessed with an Adeno-X Rapid Titer Kit (Clontech) and expressed as plaque-forming units (pfu).

In Vitro Gene Delivery

HeLa cells were infected with Adex-IL18bp or Adex-LacZ (80 MOI) in DMEM with 10% fetal-calf serum (Gibco, Rockville, MD) and incubated for 48 h at 37°C. The supernatant was collected and stored at –70°C until the biologic activity assessment.

In Vitro Assay of Biologic Activity of IL-18bp

An assay of the biologic activity of IL-18bp in the Adex-IL18bp construct was performed. IL-18bp biologic activity was considered to be represented by the ability of IL-18bp to inhibit production of IFN-γ as determined by the following method. Freshly isolated splenocytes from Lewis rats (2.5×10^6 cells/mL) in RPMI with 10% fetal-calf serum were stimulated with recombinant rat IL-18 (10 ng/mL; Pierce Biotechnology, Rockford, IL) with or without supernatant (various volumes) of Adex-IL18bp-infected HeLa cells. Supernatant of Adex-LacZ-infected HeLa cells or uninfected HeLa cells was used as the control. Clarified supernatant samples of splenocytes were assayed for rat IFN-γ by using a sandwich-type enzyme-linked immunosorbent assay (eBioscience, San Diego, CA).

Rats

Adult male Lewis rats ($RT1^l$) weighing 200–300 g were used as liver transplant recipients, and adult male ACI ($RT1^a$) rats weighing 200–250 g were used as donors. All rats were cared for and used in accordance with institutional guidelines of Keio University School of Medicine. Rats were allowed to become acclimated to the animal research laboratory for at least 5 d before they were used in an experiment, and they had free access to food and water before and after the experiment.

In Vivo Gene Delivery

To accomplish gene delivery in donor rats, 1×10^9 pfu of Adex-IL18bp or Adex-LacZ (control) in 2 mL of normal saline was injected through a penile vein 2 d before OLTx. Two days after the viral infection, the whole liver was recovered as a liver graft.

Transplantation Procedure

Operations in both donors and recipients were done with the animals under isoflurane anesthesia. OLTx was carried out by using the technique described by Kamada and Calne [14], without hepatic artery reconstruction. Livers were perfused with 20 mL of lactated Ringer's solution (4°C) through a catheter placed in the abdominal aorta, and excised grafts were stored in lactated Ringer's solution

(4°C). The cold ischemia time was less than 90 min. The anhepatic phase was less than 16 min. No immunosuppressive agents were administered. Previous studies found that the median survival time of liver allografts from ACI donors in Lewis recipients was 10 d [15–18].

Blood Sampling and Tissue Preparation

Seven days after OLTx, whole blood was collected from the infrarenal inferior vena cava of recipient rats immediately after abdominal incision. Centrifugation was used to separate out the serum, which was stored at –70°C until determination of serum alanine aminotransferase (ALT) concentrations. Concurrently, the rats were sacrificed and whole liver tissues were removed. Half the tissues were immediately frozen in liquid nitrogen; the rest were fixed in 10% formalin. Frozen tissue was stored at –70°C until the cytokine and chemokine assays.

Real-time Quantitative PCR Analysis

Total RNA was extracted from rat liver tissue by using Trizol reagent (Invitrogen, Carlsbad, CA) and purified with an RNeasy kit (Qiagen, Valencia, CA). First-strand cDNAs were synthesized from 1 μg total RNA by using oligo-dT primers (Qiagen) and ReverTra Ace RT (Toyobo, Osaka, Japan) according to the manufacturer's instructions. Real-time quantitative PCR analysis was performed with 1 μg cDNA, 300 nM primers, and a FastStart Universal SYBER Green Master Mix device (Roche Applied Science, Mannheim, Germany) using a 7500 ABI platform (Applied Biosystems, Foster City, CA). The cycling conditions were as follows: degradation of the preamplified templates at 50°C for 10 min, denaturation at 95°C for 10 min, and 50 cycles of denaturation at 95°C for 15 s, annealing at 60°C for 15 s, and amplification at 72°C for 1 min. This was followed by a dissociation step or melting-curve analysis to determine the melting point of the double-stranded DNA products produced.

Histologic and Immunohistochemical Analyses

Liver tissues were studies histologically. From formalin-fixed specimens embedded in paraffin, 5 μm-thick sections were cut and stained with hematoxylin and eosin. To evaluate the expression of IL-18bp in Adex-IL18bp-infected liver grafts, immunohistochemical studies were performed with untreated liver samples and Adex-LacZ-infected liver grafts used as controls. Mouse anti-His-tag monoclonal antibody (MBL, Tokyo, Japan) was used as the primary antibody (1:1,500 dilution; 120 min at room temperature). Alexa Fluor 488 goat anti-mouse antibody (1:1,600 dilution; 60 min at room temperature; Invitrogen, Tokyo, Japan) was used as the secondary antibody. All histologic characteristics were assessed by a pathologist.

Statistical Analysis

Student's t-tests were used to compare results (means ± SEM) in two groups. Allograft survival times in the treatment and control groups were compared by using log-rank testing. A P value <0.05 was considered to represent a significant difference.

RESULTS

Adex-IL18bp Vector was Functional *In Vitro*

Supernatant of HeLa cells infected with Adex-IL18bp, which should contain a considerable amount of IL-18bp, decreased IFN-γ levels in the rat splenocyte supernatant in a dose-dependent manner, indicating blockage of the activity of rat recombinant IL-18 (Fig. 1). Supernatant of Adex-LacZ-infected HeLa cells or uninfected HeLa cells had no effect on IL-18 activity (data not shown).

IL-18bp was Overexpressed in Livers of ACI Rats Treated with Adex-IL18bp

Samples of liver tissue obtained from untreated ACI rats and from ACI rats 2 d after one intravenous injection of 1.0×10^9 pfu of Adex-LacZ or Adex-IL18bp were analyzed for IL-18bp mRNA expression by using real-time quantitative PCR assays and for IL-18bp expression by means of immunohistochemical examination.

IL-18bp mRNA levels were higher in the Adex-IL18bp group than in either of the other two groups (Fig. 2). In addition, His-tag immunohistochemical analysis showed strong and clear staining in the cytoplasm of liver cell samples from the Adex-IL18bp group. Liver cell samples from the other two groups showed no staining (Fig. 3).

Expression of IL-18 mRNA was Up-Regulated in Liver Allografts 7 D after OLTx

Samples of liver tissue were obtained from Lewis recipients 7 d after OLTx of livers from ACI donors given an intravenous injection of Adex-LacZ (1.0×10^9 pfu; control); from ACI rats 2 d after one intravenous

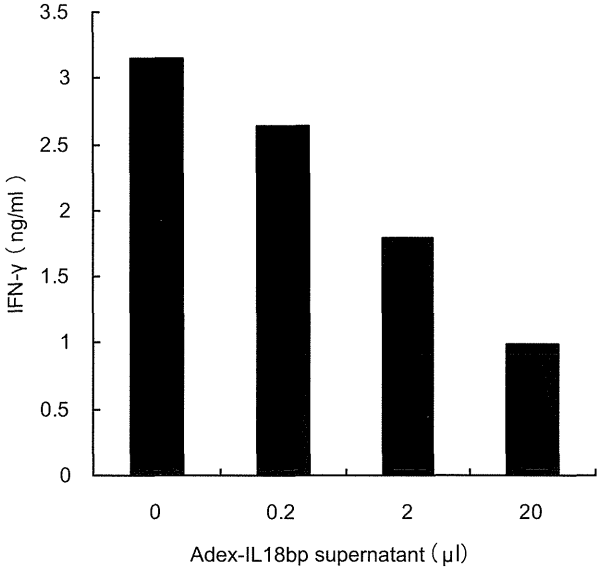

FIG. 1. *In vitro* assay of biologic activity of IL-18bp in Adex-IL18bp construct. HeLa cells were infected with Adex-IL18bp (80 MOI) for 48 h at 37°C. Freshly isolated splenocytes from Lewis rats (2.5×10^6 cells/mL) were stimulated with 10 ng/mL of recombinant rat IL-18 in various volumes of supernatant of Adex-IL18bp-infected cells. The concentration of IFN-γ in the rat splenocyte supernatant was assessed by using an ELISA. The results indicate that supernatant of HeLa cells infected with Adex-IL18bp blocked the activity of rat IL-18 in a dose-dependent manner.

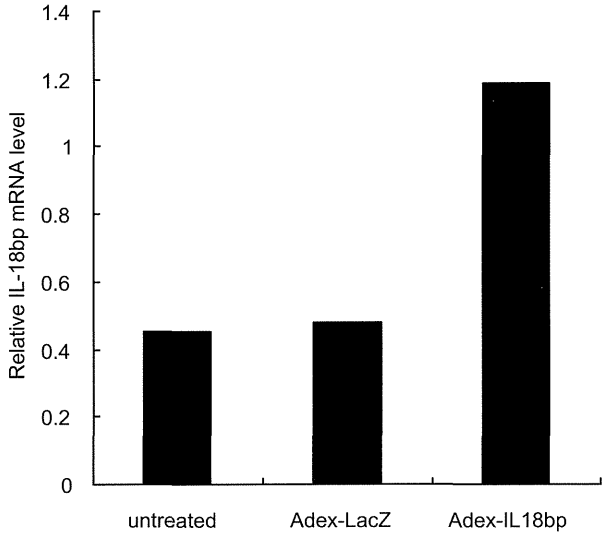

FIG. 2. *In vivo* IL-18bp mRNA assay after administration of Adex-IL18bp. Samples of liver tissue were obtained from untreated ACI rats and from ACI rats 2 d after one intravenous injection of 1.0×10^9 pfu of Adex-IL18bp or Adex-LacZ (control). Expression of IL-18bp mRNA was assessed by using quantitative real-time PCR assays, with normalization according to β-actin (housekeeping gene) expression levels. Samples from rats given Adex-IL18bp had greater IL-18bp mRNA expression than those from untreated or Adex-LacZ-treated rats.

injection of 1.0×10^9 pfu of Adex-LacZ; and from native livers in ACI rats. Levels of IL-18 mRNA in the samples were assessed by using real-time PCR assays. IL-18 mRNA levels were significantly higher in the allograft group than in either of the other two groups (Fig. 4), suggesting that the adenovirus itself did not induce IL-18 mRNA expression.

IL-18bp Overexpression Improved Liver Function after OLTx

To determine whether IL-18bp expression affected liver allograft function, serum levels of ALT were measured in Lewis recipients of ACI livers 7 d after OLTx. Histologic studies of liver allografts were performed at the same time point. As shown in Fig. 5, serum ALT levels in recipients whose donors were pretreated with Adex-IL18bp were significantly lower than those in recipients whose donors were given Adex-LacZ, the control vector (96.5 ± 12.1 *versus* 268.3 ± 99.4 IU/L; $P < 0.05$). Histopathologic analyses showed that allografts from the Adex-IL18bp group had no bridging necrosis (Fig. 6A), whereas those from the Adex-LacZ group did have necrosis (Fig. 6B). The portal vein in allografts after Adex-IL18bp pretreatment of donors was patent (Fig. 6A), whereas that after Adex-LacZ administration was severely stenosed and showed extensive infiltration with inflammatory cells (Fig. 6B).

IL-18bp Overexpression Reduced Levels of Some Chemokines and IFN-γ in Liver Allografts

Total RNA was isolated from liver allografts 7 d after OLTx and analyzed by using real-time quantitative PCR assays. Levels of CXCL10, CX3CL1, and IFN-γ mRNA were significantly lower in allografts after donor pretreatment with Adex-IL18bp compared with Adex-LacZ (Fig. 7A, B).

IL-18bp Overexpression Prolonged Allograft Survival

To investigate the possible overall benefit of IL-18bp gene delivery before OLTx, animal survival after OLTx was evaluated. As shown in Fig. 8, recipients of liver allografts from donors pretreated with Adex-IL18bp survived significantly longer than recipients whose donors were given Adex-LacZ (mean survival time, 13 *versus* 10 d).

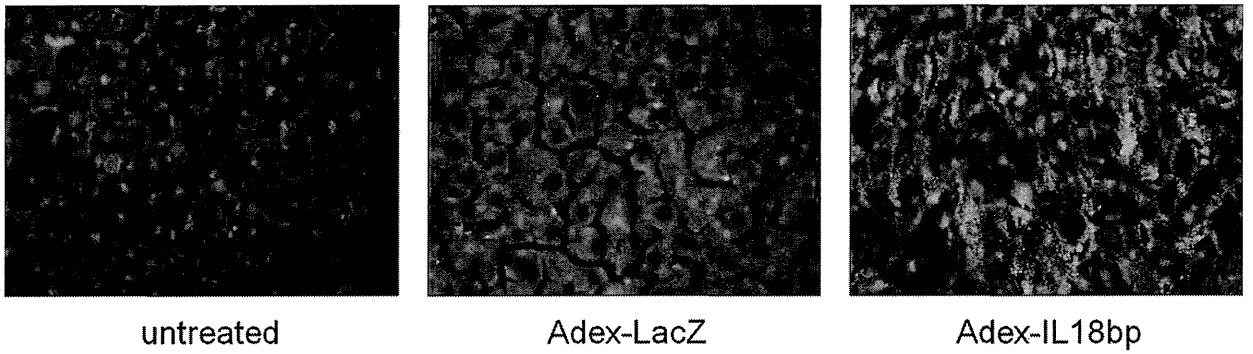

FIG. 3. Results of immunohistochemical analysis for IL-18bp linked to a His-tag in livers of donor ACI rats. Samples of liver tissue were obtained from untreated ACI rats and from ACI rats 2 d after one intravenous injection of 1.0×10^9 pfu of Adex-LacZ (control) or Adex-IL18bp. In samples from rats given Adex-IL18bp, the cytoplasm of hepatocytes was clearly stained, indicating that the Adex-IL18bp injection led to the overexpression of IL-18bp in liver grafts. (Color version of figure is available online.)

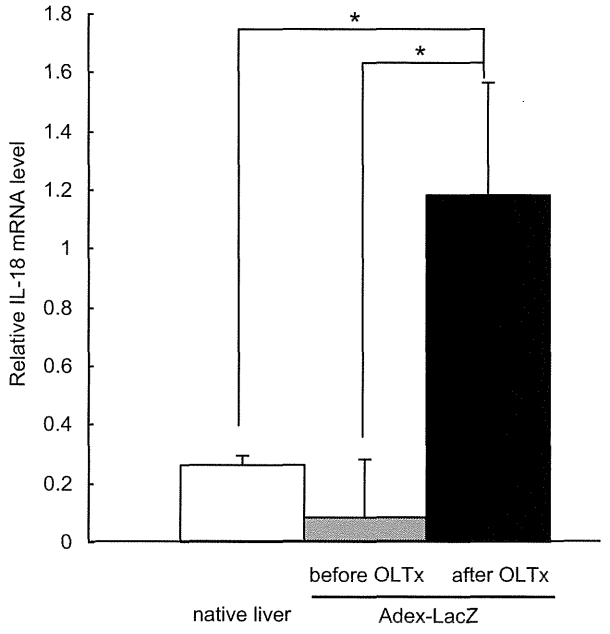

FIG. 4. Expression of IL-18 mRNA in liver allografts. Samples of liver tissue were obtained from Lewis rat recipients 7 d after transplantation of liver allografts from ACI donors given an intravenous injection of 1.0×10^9 pfu of Adex-LacZ; from ACI rats given an intravenous injection of 1.0×10^9 pfu of Adex-LacZ (no transplantation); and from native livers of ACI rats. Expression of IL-18 mRNA was assessed by using quantitative real-time PCR assays, with normalization according to β-actin expression levels. IL-18 mRNA levels were significantly higher in the allograft group than in either of the other two groups. Results represent the mean ± SEM ($n = 3$ or 4 per group); $^*P < 0.05$.

DISCUSSION

IL-18 was first cloned as an IFN-γ-inducing factor that augmented natural killer (NK) activity in splenocytes in mice [19]. Although IL-18 is structurally homologous to IL-1, and its receptor belongs to the IL-1R/Toll-like receptor superfamily, the function of IL-18 is known to be quite different from that of IL-1. Several lines of evidence suggest that IL-18 may be important in the pathogenesis of inflammatory processes. Serum levels of IL-18 have been found to be elevated in patients with postoperative sepsis [20], chronic liver disease [21–25], and acute coronary syndromes [26]. In a murine model of cardiac transplantation, IL-18 production was correlated with a histologic rejection pattern and with induction of IFN-γ [27]. Although in a previous study, levels of IL-18 mRNA in liver allografts (ACI to Lewis) peaked the d 5 after transplantation whereas liver isografts (Lewis to Lewis) had no such peaks [28], the effect of inhibition of IL-18 mRNA on acute cellular rejection (ACR) remains unclear. An investigation in a murine model of acute kidney rejection showed that IL-18 was up-regulated and produced principally by intragraft macrophages [29], and this finding is consistent with results of studies in patients [1] that established a link between kidney rejection and serum IL-18 levels.

To investigate the role of IL-18 in liver transplantation, as well as the possible effects of inhibition of IL-18 on liver allograft rejection, we constructed an adenoviral vector expressing the rat IL-18bp gene with His-tag at the C-terminal, assuming that this construction would specifically suppress IL-18 biologic activity. Gene delivery systems using adenoviral vectors have been described in several reports [30, 31], and we previously demonstrated that protein expression in rat livers after gene delivery with this method continued for more than 14 d [32]. This was sufficiently long for the current study because ACR is generally considered to occur early after OLTx. Our experiments showed that IL-18bp in the Adex-IL18bp construct had biologic activity *in vitro*. Adex-IL18bp also induced mRNA expression of IL-18bp and protein expression of His-tagged IL-18bp *in vivo*. These results indicate that Adex-IL18bp infection provided adequate IL-18bp expression. In addition, in our rat model, expression of IL-18 mRNA was up-regulated in liver allografts

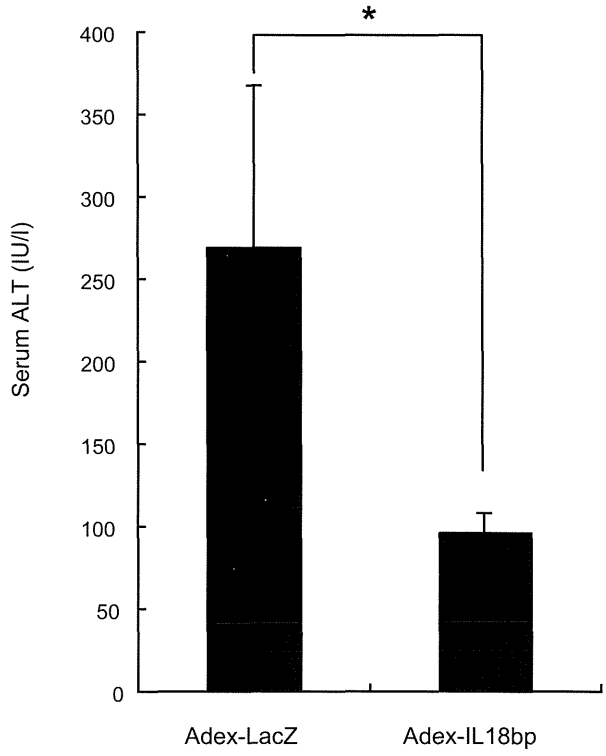

FIG. 5. Serum levels of ALT in recipients of liver allografts from pretreated and control donors. Donor ACI rats were treated with one intravenous injection of 1.0×10^9 pfu of either Adex-IL18bp or Adex-LacZ (control) 2 days before OLTx of their livers into Lewis rat recipients. Seven days after OLTx, serum ALT levels in recipients whose donors were given Adex-IL18bp ($n = 4$) were significantly lower than those in recipients whose donors were given Adex-LacZ ($n = 3$). Results represent the mean ± SEM; $^*P < 0.05$.

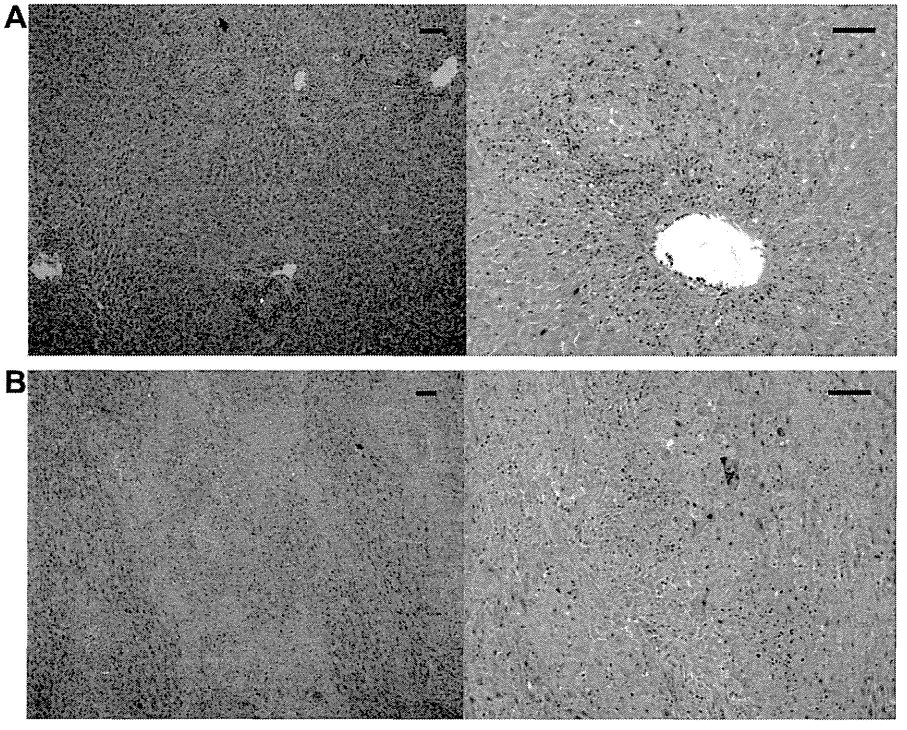

FIG. 6. Histopathologic findings in liver samples obtained from rat transplant recipients 7 d after OLTx. Samples obtained from allograft recipients whose donors were pretreated with Adex-IL18bp showed no bridging necrosis (A, left panel), whereas samples from recipients whose donors were given Adex-LacZ did show necrosis (B, left panel). The portal vein in allografts after Adex-IL18bp pretreatment was patent (A, right panel), whereas that in allografts after Adex-LacZ administration was stenosed and showed extensive infiltration with inflammatory cells (B, right panel). Hematoxylin and eosin stain; scale bar = 100 μm. (Color version of figure is available online.)

during rejection but not in Adex-LacZ-treated livers that were not transplanted. This suggests that adenoviral vector infection alone did not affect IL-18 expression.

IL-18 is known to induce IFN-γ production, and we previously showed that IFN-γ, which is produced by NK cells early after rat OLTx, is a key modulator of ACR [5]. However, a network of many cytokines is involved in ACR, so controlling rejection by inhibiting only one cytokine has been unsuccessful. To our knowledge, only one previous study found that controlling one type of cytokine alone (without any other immunosuppressive agents) suppressed ACR after allogeneic liver transplantation [33]. Our findings suggest that IL-18bp gene delivery to liver allografts may by itself significantly increase graft survival times relative to control values in a high-responder model, even though the mean difference between survival times was only 3 d. Furthermore, the lower serum ALT levels 7 d after OLTx in recipients whose donors were given Adex-IL18bp compared with Adex-LacZ clearly indicate that liver function was better after Adex-IL18bp pretreatment. The existence of this cytoprotective effect was confirmed by histopathologic studies. IFN-γ expression was also significantly decreased in the Adex-IL18bp group relative to the Adex-LacZ group. Because IL-18 is capable of inducing IFN-γ, and IFN-γ is one of the candidates for a key regulator of ACR, the cytoprotective effect resulting from the specific suppression of IL-18 may be produced through the inhibition of IFN-γ. These findings suggest that IL-18 may play an important role in liver allograft rejection. Future studies of this issue should investigate the mechanism of IL-18 in ACR by using a subtherapeutic dose of cyclosporine A with Adex-IL18bp.

To elucidate the mechanism of the effect of IL-18bp gene transfer on liver allografts in our model, we analyzed the levels of several chemokines. Chemokines are important in the trafficking of lymphoid cells to areas of inflammation, including ACR sites. Previously, we found that both CXCL10 and CX3CL1, which are induced by IFN-γ [10], were significantly up-regulated in rat liver allografts compared with syngeneic grafts beginning 3 d after transplantation [5]. These results suggest that chemokines are involved in promoting recruitment of effector cells to allogeneic tissue. Other studies showed that CXCL10 and CX3CL1 are up-regulated during rejection of murine cardiac allografts [11, 12]. Therefore, expression of CXCL10 and CX3CL1 in solid-organ allografts may be important in directing the recruitment of effector cells, including antigen-activated T and NK cells, into those allografts

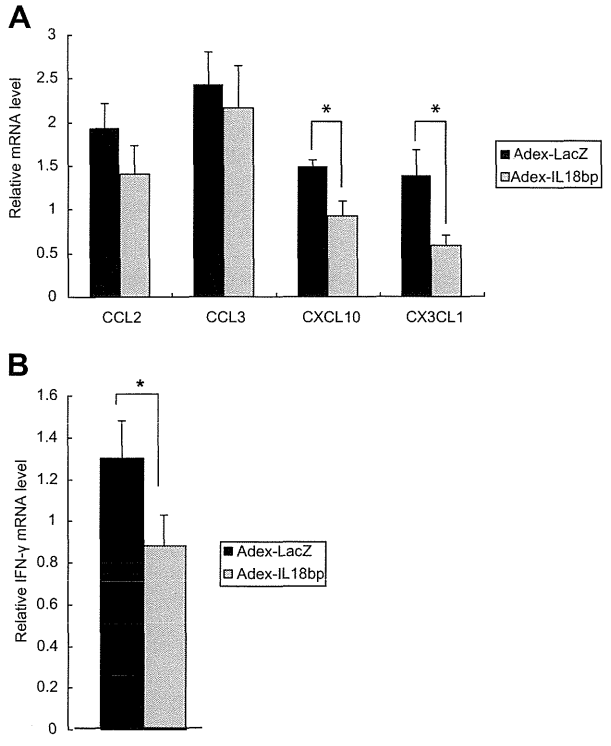

FIG. 7. Expression of chemokines and IFN-γ mRNA in liver samples obtained from rat transplant recipients 7 d after OLTx. Total RNA was isolated from liver allografts obtained from recipients whose donors were pretreated with Adex-IL18bp or Adex-LacZ (control) and was analyzed by using real-time quantitative PCR assays, with normalization according to β-actin mRNA expression levels. (A) Levels of mRNA of four chemokines in the treatment ($n = 4$) and control ($n = 3$) group. Expression of CXCL10 and CXC3L1 was significantly decreased in the Adex-IL18bp group compared with the Adex-LacZ group. (B) Levels of IFN-γ mRNA. IFN-γ expression was significantly reduced in the Adex-IL18bp group ($n = 4$) compared with the Adex-LacZ group ($n = 3$). Results represent the mean ± SEM; $^*P < 0.05$.

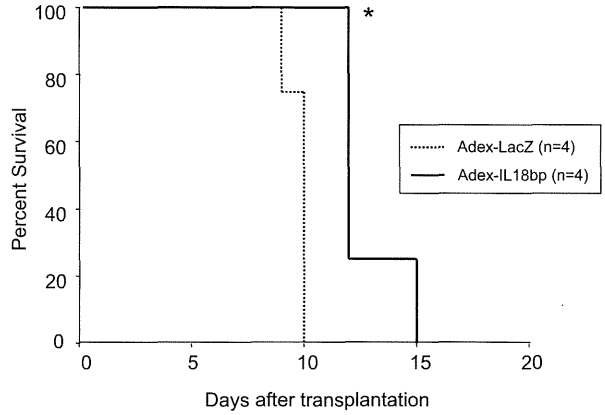

FIG. 8. Allograft survival after OLTx. Recipients of allografts whose donors were pretreated with Adex-IL18bp had significantly prolonged survival compared with those whose donors were given Adex-LacZ ($n = 4$ in each group); $^*P = 0.01$.

during acute rejection. In the current study, levels of both CXCL10 and CX3CL1 in the Adex-IL18bp group were significantly suppressed compared with control values, indicating that specific suppression of IL-18 during allograft rejection may have a crucial role in ACR through these chemokines.

Conversely, in a murine model, Wyburn *et al.* [34] found that IL-18 deficiency and IL-18 neutralization by IL-18bp did not provide significant protection against kidney allograft rejection, although IL-18 pathways appeared to be active in the alloimmune process. Anti-IL-18 strategies alone were apparently inadequate for overriding the T-cell-driven alloimmune response. However, it remains possible that in liver transplantation, a mechanism that works though IL-18 may be important in the rejection process.

There is considerable evidence showing that IL-18 has a major role in the pathogenesis of inflammation or the alloimmune response [1, 20, 21, 26, 35-39]. We previously reported that mitigation of nonspecific inflammation, including ischemia-reperfusion injury, significantly suppresses the alloimmune response [5]. In the current study, although specific inhibition of IL-18 significantly prolonged allograft survival, the difference in survival time was not large. Furthermore, whether this inhibition suppressed nonspecific inflammation or a specific immune response—that is, ACR—is unclear. Additional studies using such methods as combination therapy including Adex-IL18bp and another agent may be needed to clarify the possible cytoprotective effects of specific suppression of IL-18 in liver transplantation.

In the light of our results, we propose a theory regarding the involvement of IL-18 and IL-18bp in liver transplantation. We found that specific suppression of IL-18 significantly prolonged allograft survival, with improvement of liver function, reduced expression of IFN-γ, and several chemokines in our model. These results suggest that IL-18 plays an important role in liver allograft rejection, through IFN-γ and chemokines, and that interventions that induce specific suppression of IL-18 may have the potential to enhance liver function early after transplantation.

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Case Report

Discontinuation of Living Donor Liver Transplantation due to Donor's Intraoperative Latex-Induced Anaphylactic Shock

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We report on a 33-year-old female liver donor candidate who developed intraoperative latex-induced anaphylactic shock during surgery for living donor transplantation. She was the mother of the organ recipient, who was a 9-year-old boy with biliary atresia. We planned extended lateral segmentectomy for her. Although we dissected the ligament around the left lobe, the systolic blood pressure suddenly dropped and her body became flushed and warm. We administered transfusion and an ephedrine injection to recover the blood pressure. Because she recovered after the treatment, we restarted the procedure. However, she went into shock again within a few minutes. We decided to discontinue the operation. Postoperative blood tests revealed an increase in IgE-RAST and basophil activation, suggesting that the anaphylactic shock was induced by latex. Because latex allergy has become a public health problem, this allergy should be kept in mind as a potential donor operation risk.

Key words: Latex allergy – Living donor liver transplantation – Anaphylactic reaction – Shock

Latex is derived from the *Hevea brasiliensis* tree indigenous to the Amazon region of South America. It can be found in many of the items used in the medical-hospital environment, such as tourniquets, catheters, urine collecting bags, tubing, and gloves. These products can be the source of reactions to latex. The first report of anaphylactic reactions linked to latex sensitivity was in 1989[1] and since then the number of case reports of latex-induced anaphylactic reactions has steadily grown. Here, we

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report a case of a living donor for liver transplantation in whom latex-induced anaphylactic shock occurred during the operation, necessitating discontinuation of both the donor and recipient operations.

Case Report

This living donor was a Japanese woman who was 153 cm tall and weighed 56 kg. She was the mother of the organ recipient, who was a 9-year-old boy with biliary atresia. She had undergone cesarean section 4 times in the past and had allergy to pollen and mackerel. Her preoperative evaluation (hemogram, biochemical markers, coagulation profile, hepatitis virus markers, tumor markers, abdominal X-ray, abdominal-enhanced computed tomography [CT], and drip infusion cholangiography CT) revealed no abnormalities, except for positivity to antinuclear antibody. At our facility, intraoperative donor liver biopsy (zero biopsy) is performed routinely at the beginning of living donor liver transplantation (LDLT) to confirm eligibility of the donor liver for LDLT in terms of steatosis and portal zone inflammation.[2] We usually perform lateral segmentectomy and extended segmentectomy through a 10-cm incision using a Thompson retractor (Thompson Surgical Instruments Inc, Traverse City, Michigan). For this donor, we planned a 10-cm laparotomy operation that included zero biopsy and subsequent extended lateral segmentectomy, estimating that the graft weight-to-recipient weight ratio was 1.31%. During the operation, we initially made a 10-cm incision in the donor's upper abdomen and performed zero biopsy of the liver, which revealed no abnormal findings. Although we dissected the ligament around the left lobe through this incision, the systolic blood pressure suddenly dropped to 50 mmHg (Fig. 1) and her face and upper extremities became flushed and warm. We stopped the surgical procedure and administered treatment consisting of transfusion and an ephedrine injection to recover the blood pressure. We knew that she had multiple prior surgical procedures without a complication and did not strongly suspect latex-induced anaphylactic shock. One of the differential diagnoses was mesenteric traction syndrome because the episode occurred while we made traction on the liver. Because her systolic blood pressure recovered to more than 80 mmHg about 20 minutes later, we restarted the procedure avoiding traction on either the liver or mesentery. However, she again went into shock within a couple of minutes. After this second episode, we suspected latex-induced anaphylactic shock, because the use of gloves was a common denominater between the episodes. We switched to latex-free gloves and removed the urinary catheter from the patient's bladder (a latex-free urinary catheter was inserted immediately after the operation). Her systolic blood pressure recovered again within about 20 minutes after a second round of treatment consisting of epinephrine, norepinephrine, chlorpheniramine, and hydrocortisone by injection. Her body looked edematous and her intraoperative chest X-ray revealed mild pulmonary edema. We decided to discontinue the donor operation. Although the recipient operation had already proceeded to laparotomy and detachment of adhesion, it was also discontinued. The only procedure that we completed in the donor was dissection of the ligament around the left lobe. At the end of the operation, the donor's serum albumin level and prothrombin time-international normalized ratio were 1.7 mg/dL and 1.59, respectively. She was admitted to the intensive care unit intubated until the following day. Although she was edematous and drowsy for a few days after she returned to the general ward, her overall condition and blood parameters gradually recovered and she was discharged 7 days after the operation. Postoperatively, the blood level of IgE-RAST (radio-allergosorbent test, BML Laboratories, Inc, Kawagoe, Japan) was elevated (10.0 ARU/mL versus upper limit of 0.34 ARU/mL) and a basophil activation test (CD203c up-regulation; BML Laboratories) showed a strong positive result (23.1% at 1:312.5 dilution; evaluation criteria were negative at $<6\%$, positive in 15% to 20%, and strongly positive at $>20\%$), suggesting that anaphylactic shock was induced by latex. Postoperatively, we asked the patient about her history again, focusing on a specific issue of latex allergy potential and found that she had experienced itching when she used kitchen rubber gloves within the past couple of months. The recipient also gradually recovered and is currently waiting to undergo deceased donor liver transplantation.

Discussion

Latex allergy was unusual until the late 1980s, but as more health care workers began using latex gloves to control infections in the 1990s, the incidence steadily increased thereafter. Anaphylactic reaction linked to latex sensitivity was first reported in 1989,[1] and since there have been numerous case reports of latex-induced anaphylactic reactions in patients with various diseases.[3–5] The number of LDLTs per-

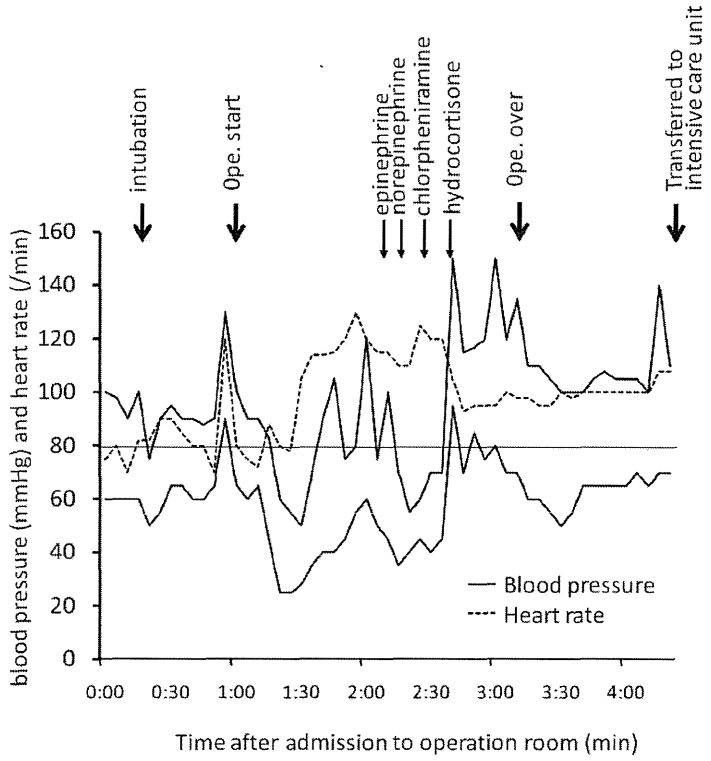

Fig. 1 Intraoperative chart of blood pressure and heart rate.

formed each year has also steadily risen since LDLT was first performed in 1988 in Brazil.[6] According to the Japanese Society for Transplantation, more than 6000 patients received living donor liver transplants between 1989 and 2009 in Japan.[7] The Organ Procurement and Transplantation Network in the United States (http://optn.transplant.hrsa.gov/) estimated 282 cases of LDLT in 2010. Although this is the first report of latex-induced anaphylactic shock occurring in an LDLT donor, it is increasingly likely that transplant surgeons will encounter donors or donor candidates with latex allergy potential.

This case highlighted the risk of latex-induced anaphylactic reaction during LDLT operation. Obstetric and gynecologic procedures are the most common settings for latex anaphylaxis during surgery,[5,8–11] but our donor's past child deliveries were all uneventful. Because she reported a recent increasingly intense allergic reaction to kitchen gloves, we assume that she had been sensitized by the kitchen rubber gloves rather than past exposure to surgical procedures, and that sensitization might have increased her risk for latex allergy during the donor operation. Preoperatively, we simply asked the patient about her allergic history but we were not focusing on latex allergy. She answered that she had allergy only to pollen and mackerel and did not refer to her recent experience of itching from kitchen rubber gloves. Because of lack of information that was indicative of the latex anaphylaxis potential, we had not considered this patient to be at risk for latex allergy. Even during the operation we did not initially suspect latex allergy, although a protocol of diagnosis and management for latex allergy and latex-free equipment store were available as part of our standard operating room equipment. Turillazzi *et al*[5] also highlighted difficulties in the initial diagnosis of latex allergy and reported a fatal case of anaphylactic latex reaction during anesthesia. Surgeons should be aware that latex allergy is rare but possible in both donor and recipient operations and should obtain detailed histories from the patients with respect to potential risk factors for latex allergy. Risk factors for latex allergy include work occupations and medical histories; health care or rubber industry workers, atopic individuals, spina bifida patients, multiple surgical procedures, and allergy to fresh fruits and nuts.[12] If a donor or recipient has such a risk factor, or, especially, has an increasing allergic reaction to rubber as did our patient, a preoperative test, such as a prick test, is recommended to confirm hypersensitivity to latex. If circulatory collapse and respiratory failure occur during surgery due to latex allergy, the donor

should be resuscitated adhering to the guideline for management of latex allergy[13] and the operation should be stopped as soon as possible. Although the implications of high doses of epinephrine for the graft have not been fully elucidated, we have no alternative but to use standard resuscitative drugs and procedures, even for the living donor.

This case also highlighted the issue of whether it is safe to use the same donor after recovery from the latex-induced anaphylactic reaction. We usually perform latex-free operations for patients with known latex allergy. If an episode of latex-induced anaphylactic reaction accidentally occurs during surgery, and if the disease the patient is being treated for is life threatening, the operation can be continued excluding latex items or the patient can be rescheduled for a latex-free operation at a later date. However, we must be always aware that donor safety is the golden rule. We cannot rule out the possibility that another allergen caused the anaphylactic reaction in this case, and it is not possible to test for allergy to all possible allergens. Even for the recipient, it is unknown whether the liver graft derived from donor with latex allergy has no immune reactions in the post-transplant course. Therefore, we ultimately decided that this woman was unsuitable as a living donor. We knew that this donor was the only possible living donor for the recipient, because all other relatives were medically or socially unqualified as living donors. However, no risk should be tolerated for the donor even if there are fewer alternative candidate donors. We believe that transplant surgeons should be knowledgeable about the risks of latex allergy during transplant surgery and use this knowledge to make more precise judgments when assessing donor qualifications.

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〔症例報告〕

術前化学放射線療法後膵頭十二指腸切除術を施行し，長期生存を認めた腹膜播種によるStage IVb膵頭部癌の1例

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要　旨：症例は70歳，男性．膵頭部癌（TS2, PV（＋），T4N0M0, Stage IVa）の診断にて術前化学放射線療法（5-FU，MMC，CDDP＋Radiation 40Gy）施行後，膵頭十二指腸切除術を行った．病理組織診断は高分化型管状腺癌 T4N0M1（PER；omentum），Stage IVbであり，病理上，腫瘍本体に加え腹膜結節にも術前化学放射線療法の組織学的効果を認めた．術後 Gemcitabine を開始したが，5年2カ月後右肺転移を認め，右肺 S9-10 切除術を施行．その後，胸膜播種を認め初回手術から6年7カ月後に死亡した．腹膜播種による Stage IVb にもかかわらず術後長期生存した1例を経験した．

索引用語：膵癌　腹膜播種　肺転移　術前化学放射線療法　長期生存

はじめに

膵癌はいまだ予後不良の疾患であり，腹膜播種による Stage IVb の長期生存例は非常に稀である．今回，我々は術後診断にて腹膜播種を認めたにもかかわらず，術前化学放射線療法および術後化学療法を行い，術後5年2カ月で肺転移に対して肺切除を施行，初回手術後6年7カ月間生存した症例を経験した．そこで術前化学放射線療法の腹膜播種巣への効果，長期生存における肺転移に対する肺切除の効果について考察した．

症　例

患者：70歳，男性．

主訴：なし．

既往歴：20歳時，肺結核にて左肺上葉2/3切除．45歳時より，心房細動（ジゴシン 0.25mg 内服中）．

現病歴：近医の血液検査で γ-GTP 高値を指摘され，腹部超音波を施行したところ膵癌を疑われたため，当院を紹介され受診した．

初回手術時現症：身長 182cm，体重 78.5kg，血圧 125/85mmHg，心拍数 56 回/分・不整，体温 36.4℃．意識清明．眼瞼結膜・眼球結膜に貧血・黄疸なし．腹部に腫瘤や表在リンパ節は触知せず．

初回手術前検査所見：

血液検査所見（Table 1）：ALT 47IU/l，γ-GTP 224IU/l と軽度肝機能障害と胆道系酵素の上昇を認めたが，TB は正常であった．腫瘍マーカーは CEA 6.0ng/ml，CA19-9 320ng/ml，DUPAN2 1390 ng/ml と上昇を認めた．

腹部造影 CT 検査（Fig. 1a～c）：膵頭部に大きさ 28×20mm の造影効果の乏しい内部不均一な腫瘤病変を認め，膵癌に矛盾しないと考えられた．前方および後方組織への浸潤が疑われ，門脈と脾静脈の合流直下に腫瘍浸潤によると思われる血管の狭小化を認めた．

腹部 MRI 検査（Fig. 2）：膵頭部に一致する主膵管は高度狭窄を示し，尾側膵管には拡張を認めた．

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Table 1　入院時血液検査所見

WBC	$7500\times10^3/\mu l$	BUN	14.0mg/dl	ALP	201IU/l
RBC	$5.52\times10^6/\mu l$	Cre	0.9mg/dl	γ-GTP	224IU/l
HGB	16.6g/dl	Na	137.9mEq/l	CH-E	264IU/l
HCT	48.8%	K	4.6mEq/l	AMY	75IU/l
PLT	$228\times10^3/\mu l$	Cl	98mEq/l		
APTT	30.8sec	Glu	151mg/dl	CEA	6.0ng/ml
PT-%	100＜%	HbA1c	7.4%	CA19-9	320ng/ml
		TC	261mg/dl	Elastase-1	177ng/ml
TP	7.1g/dl	CRP	0.30mg/dl	DUPAN2	1390ng/ml
ALB	4.2g/dl	LDH	168IU/l		
TB	0.7mg/dl	AST	32IU/l		
		ALT	47IU/l		

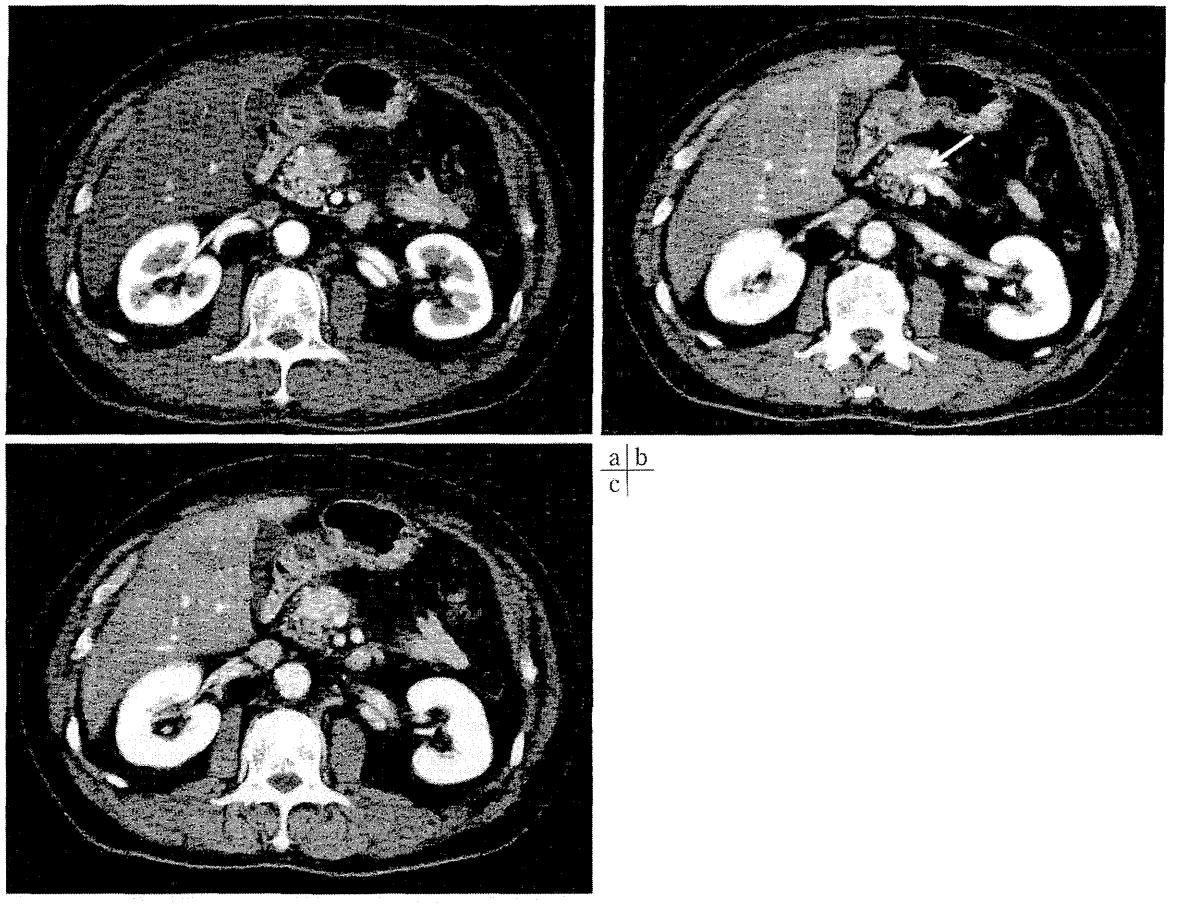


Fig. 1　腹部造影 CT 検査（a：動脈相，b，c：門脈相）
膵頭部に 28×20mm の腫瘍を認め，前方組織と後方組織への浸潤を認めた．また，門脈から脾静脈への分岐直後に腫瘍の浸潤による狭小化（矢印）を認めた．

以上より，膵頭部癌（TS2，CH（−），DU（−），S（+），RP（+），PVsm（+），A（−），PL（−），OO（−），T4, N0, M0, Stage IVa）の診断にて術前化学放射線療法を施行した．放射線療法は 40Gy（2.0Gy/day，days 1-5/w×4 計 20 回）照射し，化学療法は 5 fluorouracil（5-FU）（300mg/day，days 1-5/w×4, civ.），mitomycin C（MMC）（4mg/body/day，days 1, 8, 15, 22, bolus iv.），cisplatin（CDDP）（10mg/body/day，days 2, 9, 16, 23, bolus iv.），heparin（6000IU/body/day，days 1-7/w×

4, civ.）を投与した（Fig. 3）．術前照射線量に関しては，Gillen ら[1]の Systematic review によれば 24～63Gy までの報告があるが，我々は 50Gy を照射した同じレジメンによる膵癌非切除例に対する経験から抗腫瘍効果と耐術能を損なわないバランスを考慮し 40Gy とした．術前化学放射線療法後，原発巣腫瘍サイズは 28.6% の縮小を認め，CA19-9 は 24.9% 低下し（250ng/m*l*），RECIST（version 1.1）基準[2]による効果判定では SD であった（Fig. 4）．術前化学放射線療法終了 1.5 カ月後に膵頭十二指腸切除術を施行した．

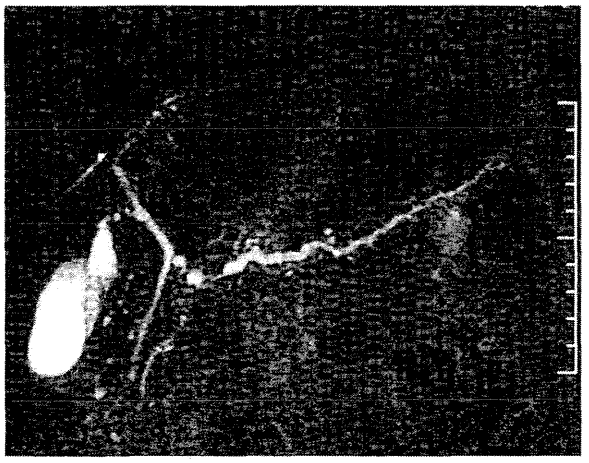


Fig. 2　MRCP 検査
膵頭部では主膵管の不整と高度狭窄，その尾側で拡張を認めた．

手術所見：最初に腹腔鏡にて遠隔転移がないことを確認し，開腹した．次に，リンパ節＃16a2，＃16b1 に転移がないことを迅速病理にて確認した．腫瘍は脾静脈合流部直下の上腸間膜静脈に浸潤していたため門脈を合併切除し，膵頭十二指腸切除術（D2 郭清，上腸間膜動脈神経叢右半周郭清）を施行した．再建は Child（IIA-2）とした．さらに，術後門注補助化学療法のために再疎通させた臍静脈よりカテーテル（6Fr；アンスロン P-U カテーテル，東レメディカル）を挿入し，先端を上腸間膜静脈内へ留置した．門注療法は術直後から 4 週間，5-FU，MMC，CDDP，heparin の多剤併用持続投与で行った[3]．

病理組織学所見：腫瘍本体部分では腺管構造の乱れの見られる部分で癌細胞が高度に変性し，マクロファージの出現も見られ，術前化学放射線療法の効果と考えられた（Fig. 5a，b）．腫瘍の変性像は 1/3 以上 2/3 以下であり，「臨床・病理乳癌取扱規約」の「組織学的効果の判定基準」を参照すると Grade 1b であった[4]．開腹時所見では腹膜播種は分からなかったが，切除検体の網嚢内の大網に腹膜播種結節を認めた．腹膜播種部には癌細胞が散見されたが，結合織・線維化が強く，小血管の閉塞像も認めた．これらから腹膜播種部にも術

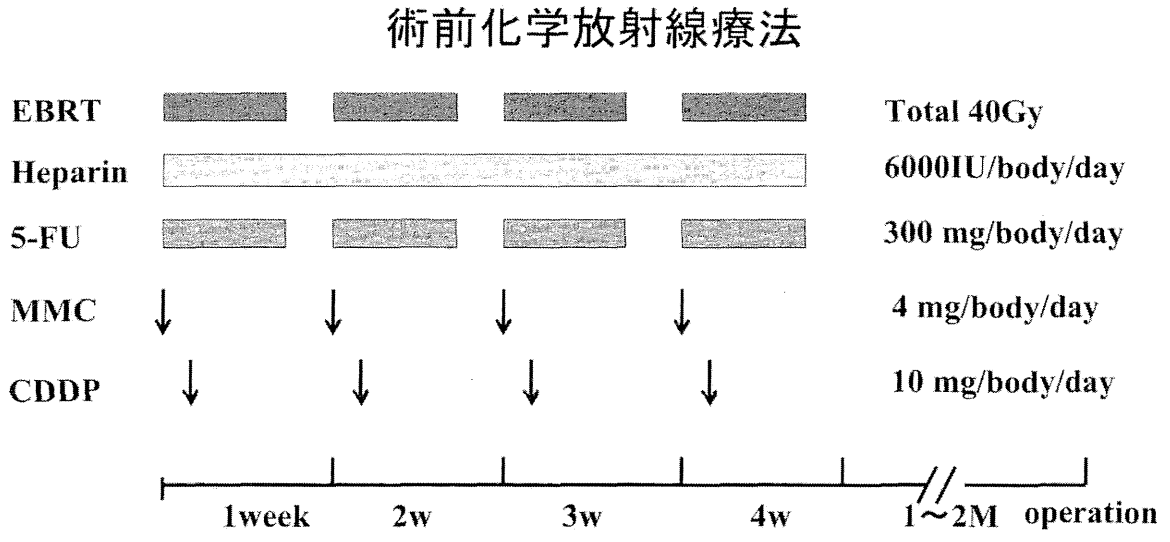


Fig. 3　術前化学放射線療法のレジメン
体外照射 40Gy に加え，5 fluorouracil，mitomycin C，cisplatin，heparin を併用投与した．
ERBT：体外照射，5-FU：5 fluorouracil，MMC：mitomycin C，CDDP：cisplatin

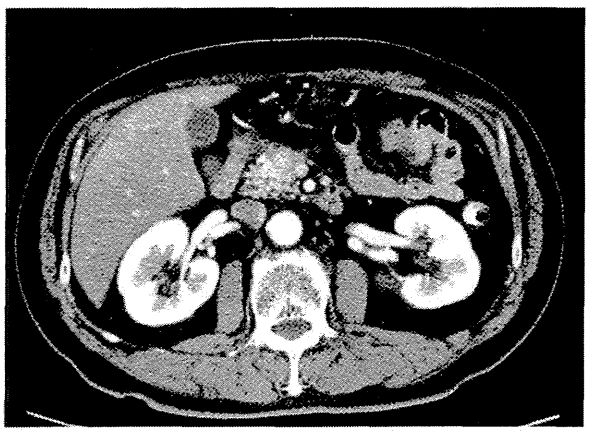
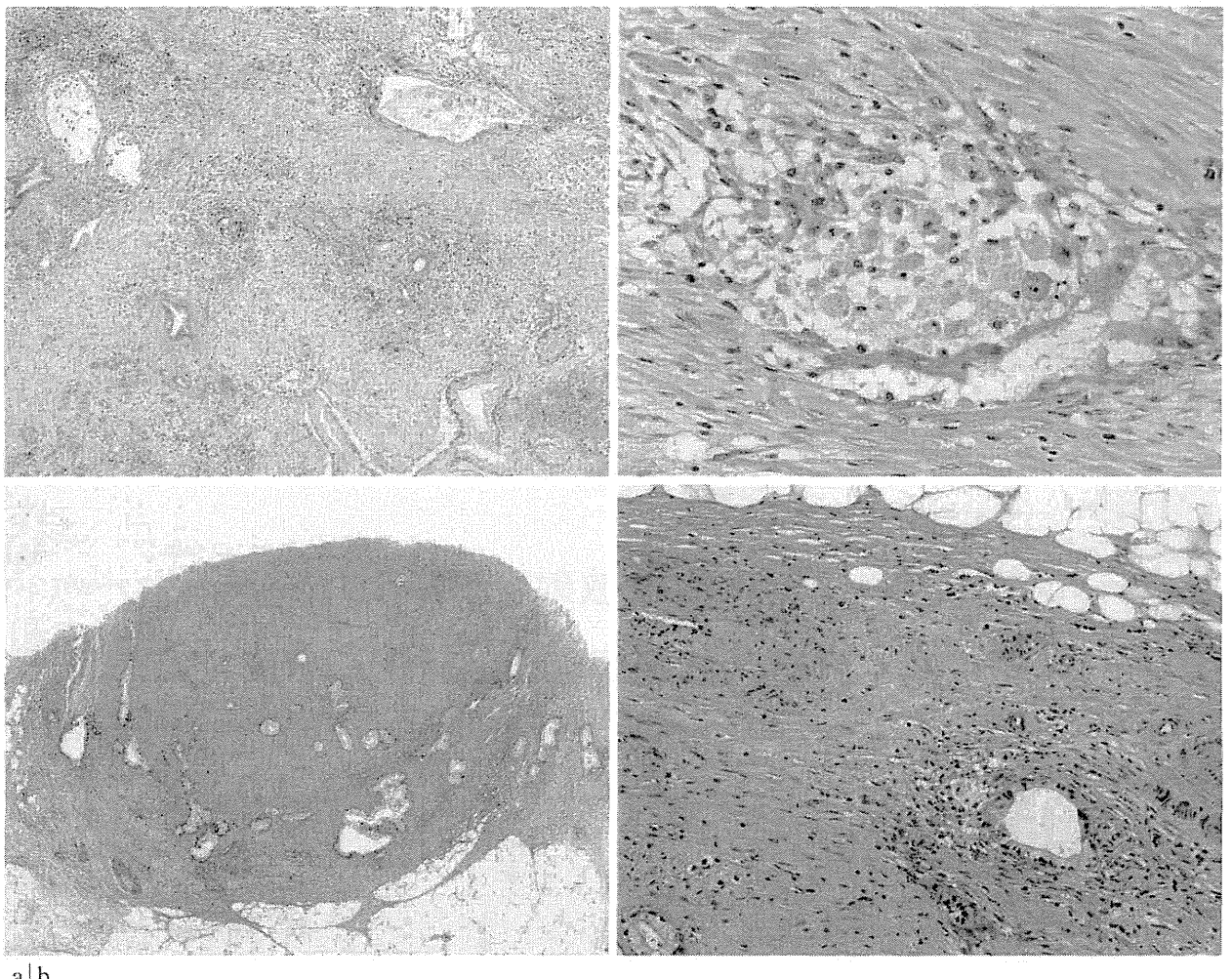


Fig. 4　術前化学放射線療法後CT検査
術前化学放射線療法後，原発巣腫瘍サイズは術前化学放射線療法前（Fig. 1）と比較して28.6%の縮小を認め，RECIST基準による効果判定ではSDであった．

前化学放射線療法の効果が及んでいる可能性が示唆された(Fig. 5c，d)．以上より，浸潤性膵管癌，Tubular adenocarcinoma，well－differentiated type，scirrhous type，INFβ，ly1，v1，ne1，mpd(－)，CH(－)，DU(－)，S(＋)，RP(＋)，PVsm(＋)，A(－)，PL(－)，OO(－)，T4，N0，M1(PER；omentum)，Stage IVb，PCM(－)，BCM(－)，DPM(－)と診断された．

術後，外来にて血清CEAの漸増を認めたため術後1年9カ月でGemcitabine（GEM）1400mg/bodyを3投1休投与にて開始した．血清CEAの上昇は持続したが画像上再発所見は認めなかっ



a｜b
c｜d

Fig. 5　病理組織学的所見（HE染色）
a：膵原発巣（×40），b：膵原発巣（×200）―腺管構造の乱れの見られる部分で癌細胞が高度に変性し，マクロファージの出現も見られ，術前化学放射線療法の効果と考えられた．c：腹膜播種巣（×40），d：腹膜播種巣（×200）―腹膜播種部には癌細胞が散見されるが，結合織が多く線維化が強く，血管の閉塞像も認める．これらから腹膜播種部にも術前化学放射線療法の効果が及んでいると考えられた．

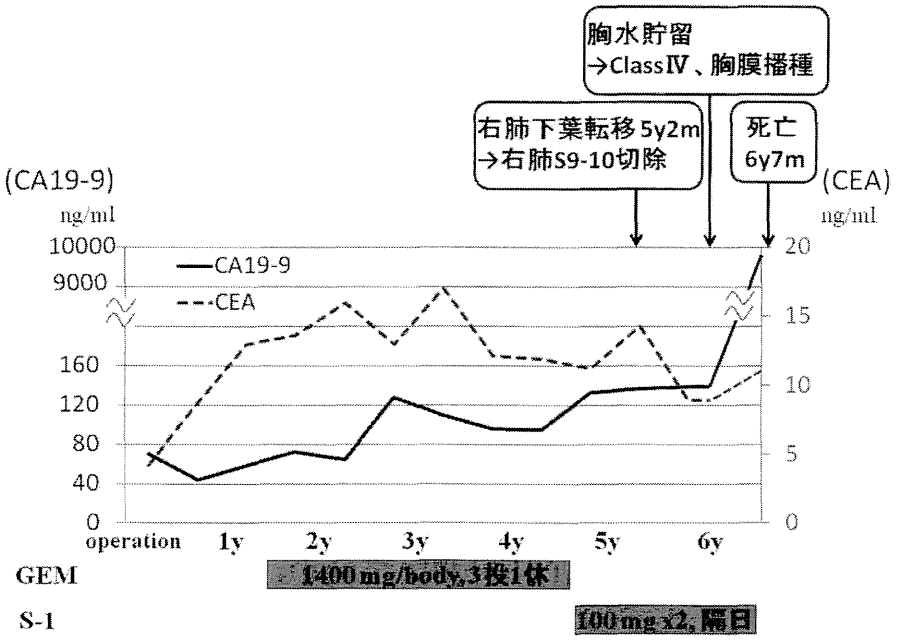


Fig. 6　術後経過と腫瘍マーカー推移
CEAの漸増を認め術後1年9カ月でGemcitabine 1400mg/bodyを3投1休投与にて開始した．画像検査上，再発は認めなかったが，術後4年9カ月でS-1 100mgに変更し治療を継続．5年2カ月のCTにて右肺下葉結節影を認め，増大傾向を認め，切除施行．その後，胸膜播種もあり6年7カ月で永眠した．

た．術後4年9カ月からはS-1 100mg/日，2分服，隔日投与（休薬期間なし）に変更し治療を継続した（Fig. 6）．

膵癌切除術後5年2カ月の胸部CTにて右肺下葉に結節を認めたため，原発性肺癌を疑い，右肺S9, 10切除施行した．しかし，肺切除標本の病理検査では，HE染色で粘液産生の目立つ腺癌の像を認め，免疫染色を施行したところ，肺腺癌に特異的なTTF-1染色は癌腺管で陰性となり，既往膵癌の肺転移と診断された．

術後S-1の投与を継続したが，胸膜播種となり，膵癌切除後6年7カ月で永眠された．

考　察

膵癌は消化器悪性腫瘍の中でも特に予後不良の疾患であり，膵癌全国登録での膵頭部癌の5年生存率は切除・非切除全症例で10.7%，切除症例でも13.0%と報告されている[5]．

膵癌術後の5年生存例は本邦でも少なく，特に膵癌腹膜播種陽性症例に対する切除後の長期生存の報告はほとんどない．本症例は術前診断が膵頭部癌Stage IVaであったために術前化学放射線療法を施行し，その後手術となった．さらに術中の腹腔鏡による遠隔転移診断も陰性であったため，膵頭十二指腸切除術を施行したが，摘出した標本にて網嚢内に播種巣を認め最終的にfStage IVbの診断となった．本症例では切除標本において腫瘍本体以外に播種巣にも術前化学放射線療法の治療効果が顕著に見られ，他に腹膜転移，リンパ節転移などの遠隔転移は認めなかった．さらに，画像所見でも明らかな腹膜再発所見は最後まで見られなかった．つまり，手術治療による切除と術前化学放射線療法の治療効果が，長期生存につながったと考えられた．また，リンパ節転移を認めなかったことも長期生存に寄与した要因の一つと考えられ，これも術前化学放射線療法の効果の可能性が考えられた．Tinklら[6]は切除可能膵癌において術前化学放射線療法によりリンパ節転移が術前と手術時で50%から32%に減少し，リンパ節転移が術後生存率において，有意な予後因子であったと報告している．Breslinら[7]も同様の報告をしている．

Table 2　本邦における膵癌肺転移切除報告例

報告者	術式	報告年	Stage	肺転移出現までの期間	再発病巣数	肺転移術式	肺切除後経過
伊藤[14]	膵全摘	2003	IVa	9年	4カ所	右S3区域切除 S6区域切除 左下葉切除	外来通院中
櫻井[15]	PPPD	2004	II	5年	1カ所	左下葉切除	4カ月生存中
島田[16]	PD	2004	IVb	5年	1カ所	左下葉切除	2年生存中
保田[17]	DP	2009	II	4年	2カ所	右下葉切除 右上葉部分切除	37カ月後永眠
保田[17]	PPPD	2009	IVa	3年	1カ所	右下葉切除	6カ月後生存中
江本[18]	PD	2010	IVa	6年	1カ所	右S8区域切除	1年生存中
自験例	PD	2010	IVa	5年4カ月	1カ所	右S9，10区域切除	17カ月後永眠

加えて本症例がStage IVbにもかかわらず長期生存が得られたのは，肝転移予防対策として施行した経門脈的持続投与による術後補助療法[3]，外来で施行した化学療法，さらには膵癌切除術後5年2カ月に出現した肺転移に対して切除したことなども長期生存に寄与した可能性が考えられた．

膵癌再発の好発部位は膵床部局所および肝臓であり，それぞれの再発率は71.8%～92%，61.5～92%と報告されている[8~11]．一方，肺転移の割合は6.4%～22%と再発部位としては比較的稀である[10,11]．肝転移再発までの平均期間は9.0カ月，局所再発では9.5カ月，多くは2年以内に起こるといわれている．相浦ら[12]の報告では，膵癌術後の長期生存例の剖検の検討で，長期生存例では肺転移の頻度が多かったとしている．Katzら[13]も同様の報告をしている．本症例においても膵癌切除後，長期経過することにより肺転移が前面に顕在化してきたものと考えられた．

医中誌による1983年～2010年までの検索では，膵癌術後肺転移に対して肺切除を行った報告は自検例を合わせて7例であった[14~18]．いずれの症例も，長期生存後の肺転移再発である（Table 2）．肺転移巣に関しても，遺残のない切除が可能な場合，積極的に切除を行うことが予後の改善につながる可能性が考えられた．

結　語

今回，我々は術前化学放射線療法後に膵頭十二指腸切除術を施行し，集学的治療を積極的に行うことにより長期生存を認めた腹膜播種によるStage IVb膵頭部癌の1例を経験した．

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Long-term survivor of stage IVb pancreatic cancer with peritoneal dissemination treated with neoadjuvant chemoradiotherapy followed by pancreaticoduodenectomy

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Key words: Pancreatic cancer, Dissemination of peritoneum, Metastasis of lung, Neoadjuvant chemotherapy, Long-term survival

We report a case of long-term survivor of stage IVb pancreatic cancer with peritoneal dissemination. A 70-year-old man with pancreatic cancer (TS2, PV (+), T4N0M0, stage IVa) underwent pancreaticoduodenectomy after neoadjuvant chemoradiotherapy (40Gy radiation + 5-FU, MMC, and CDDP). Pathological examination of the resected specimen revealed a T4N0M1 (PER; omentum), Stage IVb tumor. In addition, neoadjuvant therapy led to further dissemination of the omental nodule and primary tumor. The patient underwent postsurgical chemotherapy with gemcitabine because of an increase in serum CEA levels. Follow-up CT performed 62 months after primary resection detected a mass in his right lung, which was treated by right lower lobectomy. Immunohistochemical study of the lung mass revealed it to be a metastatic lesion of the primary pancreatic tumor. Seventeen months after lung surgery, the patient died because of pleural dissemination of the pancreatic cancer.

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連載
第6回
消化器領域における再生医工学

脱細胞化肝骨格を用いた肝臓再生

八木　洋*　北川雄光*

Summary

肝再生医療開発へのニーズは，近年の幹細胞技術の発展とともに急速に高まっている．肝臓微細環境を再生するためにこれまでさまざまな医用工学的手法が開発されてきたが，われわれはそのなかでも，最近になり臓器全体を対象として発表された脱細胞化技術に着目し，これを肝臓に適応した．本技術はこれまでの医用工学的手法を用いた治療法と比較して，臓器特異的細胞外マトリックス（ECM）と三次元微細構造を保ちながら，大血管まで連続する移植可能な構造を有するといった特徴を備えている．この技術に現在発展の著しい胚性幹細胞（ES細胞）/人工多能性幹細胞（iPS細胞）技術を応用することで，臨床応用可能な新しい技術として発展する可能性が期待される．

Key words

細胞外マトリックス（ECM），幹細胞（stem cell），肝細胞，三次元構造

はじめに

肝臓は切除しても自己再生する大変ユニークな臓器の1つとして認識されていながら，切除後の再生とその制御のメカニズムに関してはいまだに不明な点が多い．それだけに組織再生技術を発展させるうえで，肝臓の自己再生能制御機構を明らかにすることは重要な課題となっている．日本だけで年間4万人以上といわれる肝疾患患者のなかで，肝不全などの関連疾患によって約1万人が亡くなっている．重症肝不全の唯一の根治的治療法は肝移植であるが，慢性的なドナー不足によって，移植医療を享受できる患者数は，移植を必要とする患者数の3割にも満たないと考えられている．したがって，この自己再生能を有する肝臓をいかに再生し，新しい治療法として応用するかという肝再生医療開発へのニーズは，近年の幹細胞（stem cell）技術の発展とともに急速に高まっている．

近年著しい発展を遂げている数々の医用工学的手法は，肝再生機構の理解を深め，臨床応用を考えるうえで大きな役割を担ってきた．とくに肝臓微細環境を再現するために，さまざまな方法が開発されてきたが，そのなかでわれわれは，以前から存在する手法でありながら臓器を対象とした新しい方法が最近になって発表された脱細胞化技術を，世界に先駆けて肝臓に適応した[1]．本手法は肝臓における組織再生を理解するうえでの基盤技

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