Plasma Irradiation Effects to Intra-Abdominal Organs Compared with Adhesion Mouse Model

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ABSTRACT: In this study, we evaluated the effects of plasma irradiation on intra-abdominal organs using low-energy atmospheric-pressure plasma (LEAPP) compared to cauterization in the mouse adhesion model. Plasma irradiation of the cecum caused an increase of inflammatory proteins, such as serum amyloid proteins, inter- α -trypsin inhibitor, and lactoferrin, in ascites. Plasma irradiation of the cecum and cauterization of the cecum induced mRNA expression of the pro-inflammatory cytokine IL-6. On the other hand, induction of lactoferrin in the cecum was lower with plasma irradiation than with cauterization. In addition, few inflammatory cells infiltrated the cecum after plasma irradiation compared with cauterization. We also demonstrated that very few adhesions formed after plasma irradiation compared to the mouse adhesion model. Furthermore, plasma irradiation is involved in IL-10 induction in blood plasma. Taken together, these results indicate that the reduced inflammatory effects of LEAPP should result in decreased formation of adhesions in tissues in the abdominal cavity.

KEY WORDS: plasma medicine, inflammation, peritoneal cavity, adhesion, ascites, proteomics

I. INTRODUCTION

Abdominal adhesion formation is an almost inevitable consequence of surgery (Fig. 1).^{1,2} Although intraperitoneal adhesion is not clearly identified without additional surgery,³ it is considered a very common outcome after surgery.^{4–7} Complications including small bowel obstruction,⁵ abdominal pain,⁶ and infertility in women⁷ are prevalent issues due to adhesion formation, and they sometimes requires inpatient hospital care, surgical intervention, or both.^{4,8} Therefore, this surgery-related event decreases the quality of life (QOL) of some patients.

Management and prevention of abdominal adhesion have attracted considerable concern by surgeons to reduce the need for later surgery due to their formation.³ When using a high frequency electric knife (HFEK) in surgery, cut mode or coagulation mode

FIG. 1: Adhesion formation is secondary event after surgery. (a) Postoperative adhesions occur after almost every abdominal surgery and are the leading cause of intestinal obstruction. Adhesion formations between small intestine and abdominal wall were confirmed after surgery of gastric cancer. (b) Laparoscopic surgery for adhesiolysis is basically performed to cut the adhesion and separate the tissues, imposing a burden on patients.

generates the heat, which denatures tissue proteins, causes hemostasis, and thermally damages tissues.^{5,9} It might lead to leakage of plasma proteins, including fibrinogen and soluble fibrin at the site, which causes adhesion formation.¹⁰ Many investigators have taken on the challenges of preventing postoperative adhesion formation,^{3,11–13} and various types of polymer sheets have been developed in an aim to prevent adhesion formation.^{14–19} Separating the peritoneal surface around the wound from the organs by sodium hyaluronate/carboxymethylcellulose has been subjected to clinical trials and provided good results.²⁰ However, location and pattern of adhesion formation are not limited to the tissue around the wound, and their development is basically unpredictable in the peritoneal cavity.³ The exact mechanism of how intraperitoneal adhesion formation is regulated is still unknown.^{3,11–13} Adhesion formation is regarded as a positive process in the wound healing to prevent invasion of infectious bacteria. In addition, adhesions may form regardless of the surgical devices used.^{21–23}

Surgical devices that do not generate heat seem to be optimal to reduce the adhesion

formation; however, such devices have not yet been developed. Low-energy atmospheric-pressure plasma (LEAPP) is a promising new tool in the medical field because of their physicochemical property,^{24–26} suggesting that it has greater potential than HFEKs. LEAPP does not generate the heat load,^{24–26} which could theoretically provide marginal thermal tissue injury. However, little is known about the effects of plasma irradiation on tissues in abdominal cavity. Moreover, the methods to identify and measure these effects have not been established. Therefore, finding the biological marker to validate the plasma irradiation effects on tissues quantitatively is the important initial step to employ LEAPP in surgical procedures. In this study, we evaluated these effects biologically using the mouse adhesion model.

II. MATERIALS AND METHODS

A. Mice

BALB/c, C57BL/6J, male or female mice aged 8 to 10 weeks (CLEA Japan, Inc.) were used. All animal experiments were performed in accordance with the guidelines of the Institutional Animal Care Committee of the University of Tokyo.

B. Mouse Model of Surgical Adhesion Formation

We anesthetized mice with 3.6% isoflurane (300 mL/min) using an aspirator (The Univentor 400 Anaesthesia Unit, Bioresearch Center Corporation, Japan) during the operation. We removed hair from the abdominal region of the mice by shaving to prevent contamination of the wound through contact with fur. Then the skin was disinfected with 70% ethanol. Then we made a small incision to open the abdomen using a small straight operating scissor. To optimize the adhesion formation model, we cauterized the abdominal wall, cecum, small intestine, or uterus, or multiple tissues using bipolar forceps (AARON 940 High Frequency Desiccator, Bovie Medical Corporation, USA). Alternatively, after conditions were optimized so that effects of cauterizing the cecum yielded reproducible results for the adhesion model, we located the cecum using blunt anatomical forceps inserted through a small incision and carefully moved it out of the peritoneal cavity so that mesenteric blood vessels would not be damaged. The cecum was cauterized using bipolar forceps and moved into the peritoneal cavity; then we closed the abdominal cavity using a surgical stapler. To confirm adhesion formation, we reopened the abdominal cavity at the indicated time points. We also performed sham surgeries and evaluated adhesion formation by adhesion score.^{27,28} Each mouse was evaluated according to the following standard scoring system: Score 0: no adhesion; score 1: filmy adhesion; score 2: more than one thin adhesion; score 3: thick adhesion with focal point; score 4: thick adhesion with plantar attachment or more than one thick adhesion with focal point; and score 5: very thick vascularized adhesion or more than one plantar adhesion.

C. Plasma Equipment and Plasma Irradiation

We performed surgery on mice as well as cauterizing tissues. We carefully moved the cecum out of the peritoneal cavity, then irradiated the cecum with LEAPP (Dr. Sakakita kindly installed, The National Institute of Advanced Industrial Science and Technology [AIST], Japan),^{24,29} at one second per irradiation for a total of three times at the indicated discharge voltages. The flow rate of helium gas was set at two standard liters per minute, and the distance between the tip of the plasma jet and cecum was fixed at 10 mm. We moved the cecum into the peritoneal cavity, then we closed the abdominal cavity using a surgical stapler. We reopened the abdominal cavity at the indicated time points to confirm whether adhesions had formed.

D. Histological Analysis

We reopened the postoperative peritoneal cavity and isolated the cecum and epididymal adipose tissue (EAT) by scissors, then put them into 10% formalin for at least 24 hours. Subsequently, those tissues were placed in 70% ethanol solution until processing. After fixation, tissues were embedded in paraffin and prepared for paraffin block by the manufacturer (GenoStaff, Japan). The tissue was cut into 5 μm sections for histological analysis. Samples were stained with hematoxylin and eosin (H&E). The pathological sections were observed by optical microscope at $\times 1$, $\times 50$, and $\times 400$ magnifications.

E. Proteomics Analysis of Ascites

We performed surgery on mice as described previously under continuous anesthesia, and we injected 1 mL phosphate buffered saline (PBS) into the closed abdominal cavity of the mice using a disposable 27-gauge needle to dilute the ascites with PBS. We massaged the abdominal region by pushing to mix well. Then we extracted 200 μL peritoneal fluid from the abdomen using a 27-gauge needle and dispensed 50 μL for analysis following the procedure. Serum albumin and immunoglobulin in dispensed fluid were removed with the Albumin & IgG Depletion Spin Trap (GE Healthcare Life Science, USA), and we prepared serum- and IgG-free samples. Furthermore, the sample was precipitated by trichloroacetic acid (TCA) for proteomics analysis. Finally, samples were trypsinized and analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) system (LTQ Orbitrap Elite, Thermo Fisher Scientific K.K., USA). All MS/MS datasets were analyzed by using Mascot software (version 2.5.1; Matrix Science Ltd., London, UK).

F. Cytokine Assay

After we performed each surgery, we collected 15 μL blood from the tail vein at the indicated time points. Blood samples were then mixed with 20 μL of 10 mg/mL EDTA

to prevent coagulation. The blood was centrifuged at 1,000 $\times g$ for 15 min at 4°C, and we harvested 15 μ L supernatant as plasma samples, which were stored at –80°C until they were used for cytokine assays. Cytokines in plasma were then analyzed using a Bio-Plex (Bio-Rad Laboratories, USA) bead-based system, which was designed to quantitate multiple cytokines in diverse matrices. We also assessed IL-6 and IL-10 cytokine levels in blood plasma individually with IL-6 and IL-10 ELISA kits (Thermo Fisher Scientific K.K., USA).

G. Quantitative PCR of Local Tissue

We sectioned the cecum at one-third of the distance from the tip where the cauterized or irradiated region was located; then samples were placed in 500 μ L RNAlater (Thermo Fisher Scientific K.K., USA) to stabilize RNA in the tissue. To isolate total RNA from cecum samples, we first removed all feces from the cecum in a dish and washed the cecum by dipping repeatedly in 6-well plates filled with PBS. Cecum samples were then homogenized on ice in Buffer RLT (Qiagen, Germany) using a Dounce homogenizer (AS ONE, Japan). The homogenate was then centrifuged at 15,000 $\times g$ for 3 min, and the supernatant was harvested as lysate including RNA. The lysate was mixed with an equal amount of 70% ethanol, and then the mixture was applied to an RNeasy spin column (Qiagen, Germany). We finally extracted total RNA according to the manufacturer's protocol (RNeasy Mini Kit, Qiagen, Germany). mRNA was reverse transcribed to cDNA with the iScript cDNA Synthesis Kit (Bio-Rad Laboratories, USA). Target genes were quantitatively amplified by CFX96 Real-Time System (Bio-Rad Laboratories, USA). The following primers were used.

IL-6 (Forward 5'-AGTTGCCTTCTTGGGACTGA-3', Reverse 5'-CAGAATTGC-CATTGCACAAC-3')

Lactoferrin (Forward 5'-GGAGCCTTGAGGTGTCTGAG-3', Reverse 5'-CCAGGTG-GCACTCCTTGTAT-3')

Cyclophilin (Forward 5'-TTCGTGCTCTGAGCACTGGAGA-3', Reverse 5'-GGACCC-GTATGCTTTAGGATGAAG-3')

H. Statistical Analysis

Data are expressed as mean $\pm$ SEM and were analyzed using GraphPad Prism 7 statistical software (GraphPad Software, California, USA). Significant difference was determined by multiple *t*-tests.

III. RESULTS

A. Stably Occurred Abdominal Adhesion Mouse Model Was Generated

Some mouse adhesion models have been reported, such as ischemic buttons to peritoneal sidewall,³⁰ the multiple-ablation model using a toothbrush,³¹ and cauterization of the cecum using the coagulation mode of bipolar forceps.²⁸ Since bipolar forceps are a generally and widely used surgical device, we chose to generate a mouse abdominal adhesion model using bipolar forceps. First, we assessed the severity of adhesion formation by adhesion score and reproducibility of adhesion formation in case of cauterizing various tissues, changing the power of the bipolar forceps, and using mice of different sexes and ages. Thus, cauterizing the cecum of two-month-old male mice yielded formation of reproducible adhesions. We set the output power of the bipolar forceps to 3 W and cauterized the cecum three times for 1 sec each time at different areas (Fig. 2[a]), then put the cecum back into the peritoneal cavity. We confirmed that adhesions formed as early as one day after surgery. Adhesion formation occurred mainly between the cecum and epididymal adipose tissue (EAT), and they remained for 5 months after cauterization (Fig. 2[b]). Body weight change was not significantly different between sham and cauterized mice for seven days after surgery (Fig. 2[c]). Adhesions that formed in our model were flaccid or intermediate adhesions and not fibrotic (Fig. 2[d]).

B. Plasma Irradiation Showed Different Effects Compared to Cauterization

Next, we applied LEAPP irradiation (Fig. 3[a]) to the cecum three times for 1 sec each at the indicated discharge voltages (Fig. 3[b,c]). These conditions yielded almost the same effects as well as cauterizing the cecum, and they were sufficient to coagulate blood. Plasma irradiation to the cecum at 14 V or 16 V discharge voltage resulted in adhesion formation with great omentum, not EAT, which was a very weak adhesion compared to those induced by cauterizing the cecum (Fig. 3[d,e]). Adhesion score was almost 0 or 1. On the other hand, there was no adhesion formation in the abdominal cavity when the voltage was set at 18 V.

C. Infiltration of Inflammatory Cells Was Observed in Plasma-Irradiated Area

To assess tissue inflammation caused by plasma irradiation, we performed histopathological examination by H&E staining of sham, cauterized cecum, and plasma-irradiated cecum. Inflammatory cells including neutrophils and macrophages strongly infiltrated the submucosal layer beneath the cauterized area (Fig. 4[b]). On the other hand, there was infiltration of a few inflammatory cells in the submucosal layer beneath the plasma-irradiated area (Fig. 4[c]). In addition, we verified inflammatory cytokine levels in blood. Among tested cytokines, pro-inflammatory interleukin-6 (IL-6) and anti-inflammatory

FIG. 2: Cauterizing cecum results in adhesion formation. (a) We optimized conditions to generate adhesion formation in mouse peritoneal cavity. The cecum was cauterized at three points by using bipolar forceps (set 3 W output power, indicated by black arrow) were tanned on the surface of cecum (white arrow) and were put back into the peritoneal cavity. (b) White arrow indicates adhesion formation between cecum and epididymal adipose tissue. Presence of adhesions was confirmed as early as one day and still confirmed at five months after operation. (c) No significant weight changes were confirmed between sham and cauterized mouse ($N = 6$). (d) Adipose tissue formed adhesions with the cecum that were firm, not fibrotic, and steady. Inflammatory cells were also confirmed in adipose tissue, especially on the adhesion formation side.

FIG. 3: Plasma irradiation on cecum demonstrates no adhesion or adhesion with omentum. (a) Low-energy atmospheric-pressure plasma (LEAPP) equipment we used was installed in our laboratory by Dr. Sakakita (AIST, Japan). Helium was used as the carrier gas. (b), (c) Plasma produced in the quartz tube and ejected from the nozzle exit into the surrounding gas was irradiated on cecum in the same manner as cauterizing. (d) Plasma irradiation set by DC voltage of 14 V, 16 V, or 18 V resulted in adhesion formation with omentum or no adhesion formation, indicating different effects of cauterizing the cecum. (e) When DC voltage was set at 18 V, no adhesion formation was confirmed. Following the experiment, we set the DC voltage at 18 V.

FIG. 4: H&E staining demonstrates the inflammatory infiltrate and plasma irradiation on tissue induced inflammatory cells not so much as cauterizing tissue. (a) No inflammatory cells were confirmed in the sham controls. (b) The inflammatory cells infiltrate in submucosa extending up to the mucosa in cauterized cecum. (c) Submucosal inflammation was present in plasma-irradiated cecum, but there were a few inflammatory cells compared to (b). Statistical data shown on the right was representative of x400 images ($N = 4$).

interleukin-10 (IL-10) showed significant changes after cauterization of cecum, as shown in Fig. 5 ($p < 0.05$). Plasma irradiation induced IL-6 at 9 hours, whereas the IL-6 concentration after plasma irradiation was significantly lower than the IL-6 level associated with cauterization at that time. IL-10 induced by plasma irradiation showed a similar trend, with IL-10 induction by cauterization at 3, 9, and 24 hours. IL-10 concentration induced by plasma irradiation was slightly higher than IL-10 concentration induced by cauterization ($p < 0.5$ at 3 hours; $p < 0.5$ at 9 hours; $p < 0.5$ at 24 hours) (Fig. 6).

D. Proteomics Analysis Identified Inflammatory Proteins in Ascites

Furthermore, we performed comprehensive proteomics analysis of ascites for identifying specific inflammatory proteins after surgery in time-dependent manner to determine whether any inflammatory mediators are released from damaged cecum. As shown in Table 1, some inflammatory proteins were identified, such as lactoferrin, serum amyloid protein, inter- α -trypsin inhibitor, serine protease inhibitor, and apolipoprotein B, compared with sham. As shown in Tables 2 and 3, there were almost the same inflammatory proteins in ascites after both plasma irradiation and cauterization.

E. Quantitative Analysis Revealed that Plasma Irradiation on Local Tissue Exerts Lower Inflammatory Effects

Lactoferrin was identified in both cauterized and plasma irradiated cecum. Lactoferrin is known to be released from neutrophils.³² As Fig. 4 shows, a number of neutrophils infiltrating the submucosa was significantly different after cauterizing the cecum compared to plasma irradiation. Therefore, we assessed mRNA expression level using cauterized cecum or plasma-irradiated cecum in time-dependent manner. We confirmed the presence of lactoferrin mRNA using specific primers as well as the inflammatory marker IL-6, as shown in blood (Fig. 6). We demonstrated that plasma irradiation to cecum induces production of IL-6 as well as cauterizing cecum, whereas plasma irradiation did not induce lactoferrin significantly, compared to cauterization to cecum (Fig. 7).

IV. DISCUSSION

Low-energy atmospheric-pressure plasma (LEAPP) has been suggested to be beneficial in wound healing^{24,33–36} and is expected to be useful as a surgical device.^{34,35,37} The latter may reduce postoperative problems, such as adhesion formation. However, the effect of LEAPP on abdominal organs is not well understood. Therefore, we evaluated the effects of plasma irradiation and compared them to the mouse adhesion model, induced by cauterization in this study. Proteomics of ascites demonstrated the elevation of inflammatory proteins in ascites after plasma irradiation of the cecum as well as cauterization of the cecum (Tables 1, 2, and 3). These results did not show obvious differences between plasma irradiation and cauterization of the cecum. On the other hand, histological examinations revealed obvious differences, including fewer infiltrated inflammatory cells

FIG. 5: IL-6 and IL-10 were induced after cauterization in plasma. Cytokine levels in plasma were assessed using Bio-Plex. Inflammatory cytokine IL-6 were induced significantly, and pro-inflammatory cytokine IL-10 was also induced after cauterization. ($N = 3$ in each experiment).

FIG. 6: IL-6 in plasma was not induced after plasma irradiation as much as in cauterization. (a) IL-6 was induced by plasma irradiation of the cecum, but the level was much lower than that after cauterization. (b) The level of IL-10 was induced by plasma irradiation as much as cauterizing the cecum. ($N = 4$ in each experiment).

in the submucosal layer of plasma-irradiated cecum. However, massive infiltration of the inflammatory cells was observed in the submucosal layer of cauterized cecum (Fig. 4). To assess the inflammation of the local tissue by plasma irradiation and cauterization, we quantitatively assessed the mRNA expression levels related to inflammation in local areas of the cecum (Fig. 7). Hence, increased mRNA level of pro-inflammatory cytokine IL-6 was observed in plasma-irradiated cecum as well as cauterized cecum (Fig. 7[a]), indicating that plasma irradiation and cauterization of the cecum induce comparable inflammatory responses. Induction of IL-6 reveals that plasma irradiation should be a causative stimulus to non-inflammatory cells, such as endothelial cells^{38,39} and fibroblasts,^{40,41} which are known to produce IL-6 in response to inflammatory stimulation. On the other hand, lactoferrin mRNA expression, known to be produced by neutrophils,⁴² was not increased by plasma irradiation compared with cauterization, which was in response to infiltrated inflammatory cells observed in submucosal layer. In contrast, the increase of IL-6 concentration in blood plasma at 9 hours after cauterization is presumed by systemic inflammatory response to cauterizing cecum and this plasma irradiation effect was weak. We also demonstrated that plasma irradiation as well as cauterization induced anti-inflammatory cytokine IL-10 in blood plasma. Of note, IL-10 induced by plasma irradiation to cecum was slightly higher than IL-10 induced by cauterization to cecum at 3, 9, and 24 hours ($P < 0.5$). These data indicated a reduced inflammatory effect of plasma irradiation compared with cauterization and suggest that cytokine balance due to plasma irradiation is involved in wound healing,^{43,44} leading to a decrease in adhesion formation.^{21,23}

TABLE 1: Peritoneal ascites were analyzed by proteomics. Proteomics of ascites from the peritoneal cavity of sham controls, treated with cauterization or plasma irradiation to the cecum, identified some inflammatory proteins. In Tables 1–3, gray protein names indicate proteins identified in both cauterization and plasma irradiation. The number indicates identified peptides.

Accession Number	Sham				Cauterization			
	0 hr	0.5 hr	4 hr	24 hr	0 hr	0.5 hr	4 hr	24 hr
A1AG2	1	1	1	1	0	1	1	4
ENOB	0	5	2	2	0	7	6	0
CHIL3	0	0	1	1	0	1	6	2
FRIH	1	5	4	1	0	0	1	2
FRIL1	0	8	1	0	0	2	0	1
G6PI	1	5	4	1	0	4	2	0
G3P	2	0	0	4	0	2	0	0
LAC2 (+1)	4	2	2	0	0	0	0	0
K1C16	0	0	0	3	0	0	0	0
K1C17	0	1	8	14	0	0	0	0
K1C42	2	1	2	5	0	4	0	0
K2C73	3	2	2	3	0	0	0	0
TRFL	0	0	4	6	0	7	33	17
MYH1 (+1)	0	3	0	0	0	0	1	0
MYH4	0	0	27	9	0	0	15	10
PGM1	0	5	1	0	0	1	0	0
S10A9	0	0	2	4	0	4	7	6
SAA1	0	0	0	0	0	9	11	9
SAA2	0	0	0	0	0	1	5	1
SAA3	0	0	0	0	0	0	0	3
TPM2	0	0	5	0	0	0	0	0
ENOA	1	2	0	6	1	1	0	0
ACE	3	1	2	0	1	0	0	0
CBPQ	1	3	3	2	1	0	1	0
CO1A1	1	2	1	0	1	0	2	3

TABLE 1: (continued)

KCRM	0	28	2	9	1	25	8	0
FBLN3	0	0	0	0	1	0	0	3
SODE	1	0	0	0	1	0	1	3
HA10	0	0	1	0	1	1	1	4
K1C14	0	0	0	3	1	0	1	0
K2C5	0	1	2	6	1	1	1	0
K2C6A	1	4	7	14	1	2	5	0
LDHA	2	4	4	1	1	4	4	1
MUP1	16	13	1	14	1	1	1	1
PI16	3	0	0	0	1	0	1	1
PGK1	0	8	1	1	1	6	1	0
KPYM	3	12	13	3	1	9	12	4
SEPP1	1	1	1	0	1	3	4	5
SAMP	1	1	0	1	1	6	6	8
QSOX1	0	0	0	0	1	3	5	4
AMY1	2	2	2	1	2	1	1	3
CRP	2	0	2	2	2	2	3	3
CAH3	2	16	7	6	2	13	9	1
KV2A7	3	2	2	2	2	2	0	0
IGJ	4	3	3	2	2	2	2	1
ITIH4	0	0	0	0	2	11	14	20
K2C1	1	1	2	2	2	3	2	1
PPIA	2	2	2	1	2	3	0	2
SAA4	4	2	2	1	2	2	4	6
TPIS	2	8	5	2	2	6	4	0
CAH2	6	4	4	2	3	6	3	4
CATB	3	1	4	2	3	1	2	3
CD5L	4	3	5	2	3	1	3	3
F13B	0	0	1	0	3	0	4	3
CS1A	1	0	1	1	3	2	2	1

TABLE 1: *(continued)*

HEP2	0	0	0	0	3	1	2	2
IGKC	6	5	4	3	3	3	2	2
MUP2	3	3	3	2	3	1	3	2
MUP3	3	2	4	2	3	1	1	0
RET4	0	0	1	0	3	0	3	2
SPA3M	2	1	1	1	3	9	16	8
SPA3N	2	0	2	1	3	18	27	21
ADIPO	4	2	4	2	4	4	4	2
A1AT1	4	2	10	2	4	14	4	5
APOB	0	0	8	0	4	14	32	17
CBPN	4	2	5	3	4	4	6	8
LUM	4	3	4	3	4	3	5	5
MUP20	5	5	5	3	4	1	2	2
PROP	8	4	8	0	4	6	9	10
PON1	4	3	5	2	4	5	5	4
ANGT	4	4	5	3	5	7	6	4
CBPB2	7	8	8	7	5	8	8	8
CO8G	2	1	3	1	5	5	4	4
GPX3	3	1	4	2	5	3	3	3
HBA	9	5	5	5	5	12	7	11
IGHA	7	3	6	1	5	2	5	5
IL1AP	2	0	3	0	5	3	4	5
AMBP	4	2	2	2	5	5	6	8
ZPI	1	2	3	1	5	7	3	4
APOA2	6	4	5	6	6	6	6	6
FA12	2	1	6	0	6	4	7	11
CO8B	4	0	6	0	6	7	11	13
ALS	5	6	5	3	6	4	7	6
ZA2G	1	0	1	0	6	3	3	6
A2AP	2	0	2	0	7	2	6	4

TABLE 1: (continued)

CLUS	5	3	7	2	7	6	8	9
ALDOA	5	17	20	7	7	20	19	6
HBB1	10	6	4	4	7	10	8	9
ITIH3	0	0	2	0	7	6	13	21
PEDF	8	6	7	3	7	5	5	7
IC1	5	1	5	2	7	4	7	6
ACTB (+1)	5	4	6	6	8	15	7	10
CPN2	2	3	6	3	8	7	6	8
CO9	2	0	1	0	8	3	5	6
CFAD	8	7	7	7	8	7	6	6
CBG	6	8	7	5	8	7	6	5
HPT	11	8	11	9	8	21	20	19
PHLD	3	1	4	2	8	8	7	10
VTNC	6	1	8	1	8	10	9	10
A1AT3	7	6	0	6	9	0	8	10
APOH	8	3	10	3	9	9	16	11
THRB	4	0	3	1	10	7	13	12
FETUB	6	3	4	4	11	8	11	10
LIFR	11	3	10	2	11	3	6	4
AMYP	6	9	7	2	11	1	5	0
APOE	8	7	13	8	12	16	22	16
CO8A	3	1	4	0	12	10	7	12
ITIH1	3	1	5	1	12	5	6	11
A1AG1	15	13	12	11	13	14	15	18
KLKB1	4	0	8	1	13	12	13	11
A1AT5	14	9	12	11	14	14	15	17
FETUA	14	7	11	5	14	15	18	16
CFAI	9	2	7	4	14	13	15	12
EGFR	9	2	9	2	14	14	14	14
ITIH2	5	0	3	2	14	12	14	17

TABLE 1: *(continued)*

MUP6	2	2	16	2	14	8	11	6
TTHY	14	15	12	13	14	15	14	15
A1AT2	45	17	14	15	16	18	47	46
CO5	7	1	17	3	16	17	16	20
IGHM	29	27	25	18	16	16	18	11
HRG	11	4	13	4	17	16	19	17
SPA3K	15	16	14	16	18	17	8	17
APOA4	18	4	18	7	20	22	24	20
KNG1	13	7	14	8	20	19	19	24
FIBG	16	11	16	11	21	17	29	0
ICA	17	7	13	5	21	19	18	23
AFAM	22	23	23	15	22	25	28	25
FIBA	28	17	21	17	26	29	39	3
GELS	13	6	12	7	26	23	16	21
FIBB	25	13	20	9	28	28	35	0
ANT3	18	14	16	15	29	28	18	22
ALBU	8	8	27	11	30	28	26	16
CFAB	26	13	24	13	32	29	34	42
APOA1	37	26	30	27	34	36	36	40
EST1C	32	29	31	27	35	33	31	29
PLMN	15	6	16	4	35	32	38	42
CO4B	3	1	8	2	36	30	27	36
HEMO	40	33	38	27	37	39	38	44
CFAH	36	19	37	17	40	40	60	57
A1AT4	17	39	37	38	42	44	17	17
VTDB	38	25	30	26	44	47	44	37
FINC	30	18	30	8	45	42	48	37
CERU	62	32	53	45	59	63	62	63
MUG1	56	37	50	36	63	69	66	66
A2M	102	77	92	77	92	102	107	106

TABLE 1: (continued)

TRFE	117	105	91	105	107	109	110	111
CO3	79	52	91	64	118	117	123	136

TABLE 2: Peritoneal ascites were analyzed by proteomics

Accession Number	Sham				Plasma (1)			
	0 hr	0.5 hr	4 hr	24 hr	0 hr	0.5 hr	4 hr	24 hr
1433Z	0	0	0	0	0	3	0	2
ACTS (+1)	0	0	2	1	0	0	3	0
ACTA (+1)	0	0	0	0	0	2	0	4
ACTB (+1)	5	4	6	6	0	15	9	19
A1AG2	1	1	1	1	0	0	0	3
A1AT3	7	6	0	6	0	0	7	0
A2M	102	77	92	77	0	0	0	0
AMY1	2	2	2	1	0	3	3	2
ANXA1	0	0	0	0	0	0	5	0
APOB	0	0	8	0	0	24	10	39
APOC3	2	2	2	2	0	3	2	3
APOD	1	0	1	0	0	3	2	3
APOM	0	0	0	0	0	3	2	3
C4BPA	2	0	1	1	0	7	4	10
CBPN	4	2	5	3	0	10	8	10
CPN2	2	3	6	3	0	12	10	13
CD5L	4	3	5	2	0	7	4	8
CHIL3	0	0	1	1	0	0	4	2
CLUS	5	3	7	2	0	7	3	8
FA10	0	0	1	0	0	2	0	3
F13A	0	0	0	0	0	2	0	4
CO1A1	1	2	1	0	0	6	0	6
CO3A1	2	0	0	0	0	0	0	4

TABLE 2: *(continued)*

C1QC	2	0	1	1	0	1	2	3
C1RA	0	0	0	0	0	3	0	4
CS1A	1	0	1	1	0	4	0	4
CYC	0	0	1	0	0	2	0	3
FBLN3	0	0	0	0	0	8	1	5
ECM1	1	0	1	0	0	6	1	5
SODE	1	0	0	0	0	1	0	6
EZRI	0	0	0	0	0	3	0	0
FRIH	1	5	4	1	0	2	0	2
FIBG	16	11	16	11	0	29	6	33
FINC	30	18	30	8	0	54	0	75
GPX3	3	1	4	2	0	6	0	7
HA10	0	0	1	0	0	10	0	10
HSP7C	0	0	0	0	0	0	0	3
H2A1F (+5)	0	0	0	0	0	3	0	3
H2B1B (+7)	0	0	0	0	0	2	0	4
H4	1	0	0	0	0	4	0	5
HABP2	0	0	0	0	0	3	0	2
HVM06	0	0	0	0	0	2	2	4
IGKC	6	5	4	3	0	2	3	2
KV2A7	3	2	2	2	0	2	1	2
LAC2 (+1)	4	2	2	0	0	2	0	2
IGJ	4	3	3	2	0	2	2	3
ALS	5	6	5	3	0	6	5	6
ITI2	5	0	3	2	0	20	11	22
ITI3	0	0	2	0	0	8	0	18
K1C14	0	0	0	3	0	1	0	0
K1C16	0	0	0	3	0	0	0	0
K1C17	0	1	8	14	0	0	0	0
K1C42	2	1	2	5	0	0	0	0

TABLE 2: (continued)

K2C5	0	1	2	6	0	0	0	2
K2C73	3	2	2	3	0	0	0	4
TRFL	0	0	4	6	0	4	23	21
MUP1	16	13	1	14	0	1	1	1
MASP1	0	0	1	0	0	3	0	2
PERM	0	0	0	0	0	0	2	3
MYH1 (+1)	0	3	0	0	0	0	0	0
PI16	3	0	0	0	0	1	1	1
PLMN	15	6	16	4	0	28	7	42
S10A8	0	0	0	0	0	1	3	2
S10A9	0	0	2	4	0	3	9	11
THRB	4	0	3	1	0	22	0	22
SAA1	0	0	0	0	0	0	5	9
SAA2	0	0	0	0	0	0	0	5
SAMP	1	1	0	1	0	1	0	5
TFR1	0	0	0	0	0	3	0	2
TKT	0	0	0	1	0	1	3	7
TPM1	0	0	0	0	0	1	0	5
TPM2	0	0	5	0	0	4	0	1
VTNC	6	1	8	1	0	3	0	11
KAD1	0	0	0	0	1	3	0	1
ADIPO	4	2	4	2	1	4	1	3
ACE	3	1	2	0	1	2	1	2
APOA4	18	4	18	7	1	22	15	28
APOC1	0	0	2	0	1	4	3	3
CRP	2	0	2	2	1	3	1	4
CAH2	6	4	4	2	1	5	3	3
CBPQ	1	3	3	2	1	2	1	2
F13B	0	0	1	0	1	11	8	10
C1QA	1	0	1	0	1	0	3	2

TABLE 2: *(continued)*

CO2	0	0	0	0	1	4	1	3
FRIL1	0	8	1	0	1	3	0	1
FIBA	28	17	21	17	1	44	7	54
GSTM1	0	0	0	0	1	3	0	0
G3P	2	0	0	4	1	2	0	5
HGFA	0	0	0	0	1	8	4	6
IGHA	7	3	6	1	1	4	5	7
ITIH1	3	1	5	1	1	13	11	19
K2C6A	1	4	7	14	1	1	0	0
LUM	4	3	4	3	1	4	4	3
MUP20	5	5	5	3	1	3	2	2
MUP3	3	2	4	2	1	2	1	2
MDHC	0	1	0	0	1	3	0	2
MBL1	1	1	2	0	1	3	4	5
MYH4	0	0	27	9	1	17	8	6
PHLD	3	1	4	2	1	15	10	14
PGM1	0	5	1	0	1	3	2	0
PGAM2	0	0	1	0	1	3	1	0
AMBP	4	2	2	2	1	6	3	7
SEPP1	1	1	1	0	1	3	4	5
SAA4	4	2	2	1	1	7	4	8
PON1	4	3	5	2	1	9	6	10
ENOA	1	2	0	6	2	3	1	5
APOE	8	7	13	8	2	21	8	18
CBPB2	7	8	8	7	2	5	5	8
FIBB	25	13	20	9	2	38	14	48
G6PI	1	5	4	1	2	4	1	4
HEP2	0	0	0	0	2	2	5	2
MUP2	3	3	3	2	2	3	2	2
MYG	0	1	0	0	2	6	0	0

TABLE 2: (continued)

ZPI	1	2	3	1	2	5	4	10
TETN	0	0	0	0	2	2	3	3
APOA2	6	4	5	6	3	6	6	6
ACTBL	1	1	0	0	3	0	0	1
CELA1	1	1	1	1	3	4	2	1
FA12	2	1	6	0	3	14	10	14
CO8G	2	1	3	1	3	6	5	6
CFAD	8	7	7	7	3	6	6	7
GRN	0	0	0	0	3	0	2	1
ITIH4	0	0	0	0	3	6	15	20
IL1AP	2	0	3	0	3	6	4	6
LDHA	2	4	4	1	3	10	3	5
TIMP1	0	0	0	0	3	0	2	1
PGRP2	0	0	0	0	3	2	4	4
PROP	8	4	8	0	3	11	5	10
SPA3N	2	0	2	1	3	7	12	27
TPIS	2	8	5	2	3	8	2	5
CTRB1	0	0	0	0	4	0	0	0
CBG	6	8	7	5	4	6	4	9
HBB1	10	6	4	4	4	9	6	7
PRVA	0	2	0	0	4	1	0	0
PPIA	2	2	2	1	4	4	2	3
PGK1	0	8	1	1	4	5	3	1
KPYM	3	12	13	3	4	11	8	13
SSLP1	0	0	0	0	4	2	5	2
TCO2	0	0	0	0	4	1	2	1
ZA2G	1	0	1	0	4	8	10	10
ANGT	4	4	5	3	5	8	9	7
CATB	3	1	4	2	5	2	5	8
HPT	11	8	11	9	5	8	9	19

TABLE 2: *(continued)*

LIFR	11	3	10	2	5	11	11	8
LYZ2	0	0	0	0	5	1	4	3
RET4	0	0	1	0	5	7	8	7
SPA3M	2	1	1	1	5	20	8	21
SPIKL	0	0	0	0	5	2	4	1
ALBU	8	8	27	11	5	30	22	24
A1AG1	15	13	12	11	6	13	10	14
CATL1	0	0	0	0	6	1	5	1
CO8A	3	1	4	0	6	15	14	18
CO8B	4	0	6	0	6	16	14	16
DNS2B	0	0	0	0	6	0	2	0
HBA	9	5	5	5	6	10	7	7
MA2B1	0	0	0	0	6	2	1	1
LIPP	0	0	0	0	6	3	2	0
ENOB	0	5	2	2	7	12	3	2
CEAMA	0	0	0	0	7	1	4	2
CO5	7	1	17	3	7	24	22	29
CO9	2	0	1	0	7	12	10	15
IGHM	29	27	25	18	7	24	20	24
ISK1	0	0	0	0	7	5	7	5
A2AP	2	0	2	0	8	11	10	13
APOH	8	3	10	3	8	17	14	18
ALDOA	5	17	20	7	8	23	15	14
MUP6 (+2)	2	2	16	2	8	13	14	13
PEDF	8	6	7	3	8	9	10	8
IC1	5	1	5	2	9	10	12	11
TTHY	14	15	12	13	9	13	11	12
B2MG	1	1	1	1	10	5	8	5
CYTC	0	0	0	0	10	2	10	1
EGFR	9	2	9	2	10	16	18	18

TABLE 2: (continued)

FETUB	6	3	4	4	10	14	14	16
PATE4	0	0	0	0	10	6	10	6
A1AT1	4	2	10	2	11	17	4	17
CAH3	2	16	7	6	11	15	7	9
CEL2A	0	0	0	0	11	3	5	3
CFAI	9	2	7	4	11	23	22	23
KLKB1	4	0	8	1	11	25	20	24
SVS5	0	0	0	0	12	6	14	7
UROK	0	0	0	0	12	1	7	0
HRG	11	4	13	4	13	19	18	24
KNG1	13	7	14	8	13	20	17	21
SVS6	0	0	0	0	13	7	13	9
A1AT5	14	9	12	11	15	20	16	19
FETUA	14	7	11	5	15	20	16	21
CO4B	3	1	8	2	15	32	17	46
A1AT2	45	17	14	15	16	43	16	46
ICA	17	7	13	5	16	31	27	33
AFAM	22	23	23	15	18	29	21	27
ANT3	18	14	16	15	18	28	24	30
KCRM	0	28	2	9	18	23	4	9
SVS4	0	0	0	0	18	12	18	13
GDN	0	0	0	0	19	0	14	0
SPA3K	15	16	14	16	20	9	22	9
APOA1	37	26	30	27	21	39	33	44
AMYP	6	9	7	2	21	23	16	14
CERU	62	32	53	45	25	61	46	63
CFAB	26	13	24	13	25	45	34	47
CFAH	36	19	37	17	26	59	46	63
GELS	13	6	12	7	26	36	33	34
VTDB	38	25	30	26	28	48	39	55

TABLE 2: (continued)

EST1C	32	29	31	27	29	34	31	34
HEMO	40	33	38	27	32	39	39	40
MUG1	56	37	50	36	35	87	71	77
QSOX1	0	0	0	0	35	12	27	10
A1AT4	17	39	37	38	39	17	41	18
PZP	0	0	0	0	52	110	102	106
CO3	79	52	91	64	66	107	98	135
TRFE	117	105	91	105	91	117	102	110

TABLE 3: Peritoneal ascites were analyzed by proteomics

Accession Number	Sham				Plasma (2)			
	0 hr	0.5 hr	4 hr	24 hr	0 hr	0.5 hr	4 hr	24 hr
6PGD	0	0	0	0	0	0	0	8
ACTS (+3)	0	0	2	1	0	0	3	2
A1AT3	7	6	0	6	0	0	9	0
A2M	102	77	92	77	0	0	0	0
ANXA5	0	2	0	1	0	3	1	2
APOC1	0	0	2	0	0	0	3	2
ENOB	0	5	2	2	0	5	4	12
CRP	2	0	2	2	0	0	2	4
CAMP	0	0	0	0	0	0	1	3
CHIL3	0	0	1	1	0	0	2	13
FA10	0	0	1	0	0	1	0	5
F13A	0	0	0	0	0	1	1	7
CO3A1	2	0	0	0	0	0	0	3
C1RA	0	0	0	0	0	1	1	4
SODE	1	0	0	0	0	1	0	3
FBLN1	0	0	0	0	0	0	1	5
HBB1	10	6	4	4	0	5	5	5
LAC2 (+1)	4	2	0	0	0	2	0	1
PLAK	0	0	0	2	0	2	0	6
TRFL	0	0	4	6	0	0	12	35

TABLE 3: (continued)

LKHA4	0	0	0	0	0	0	0	5
MASP1	0	0	1	0	0	1	1	4
MBL2	0	0	0	0	0	2	1	3
PERM	0	0	0	0	0	0	0	4
MYG	0	1	0	0	0	7	3	5
MYL1	0	0	0	0	0	0	0	4
MYH1	0	3	0	0	0	0	4	0
MYH4 (+1)	0	0	27	9	0	0	49	28
PGRP2	0	0	0	0	0	1	1	3
NGP	0	0	0	0	0	0	0	6
NUCB1	0	0	1	0	0	1	0	4
PRVA	0	2	0	0	0	2	2	6
GP1BA	0	0	0	0	0	1	1	4
S10A8	0	0	0	0	0	0	4	3
S10A9	0	0	2	4	0	0	11	7
RNAS4	0	0	0	0	0	0	0	3
SAA1	0	0	0	0	0	0	0	6
TETN	0	0	0	0	0	1	1	3
TALDO	0	0	0	0	0	0	1	5
TPM1	0	0	0	0	0	3	12	3
TPM2	0	0	5	0	0	1	4	6
ENOA	1	2	0	6	1	0	0	3
ANXA2	0	0	0	0	1	0	0	4
APOB	0	0	8	0	1	14	7	45
B2MG	1	1	1	1	1	4	2	2
CALM	0	0	0	0	1	1	1	3
CHLE	0	0	0	0	1	3	1	5
F13B	0	0	1	0	1	4	3	9
COF1	0	0	0	0	1	0	0	3
C1QA	1	0	1	0	1	1	1	3
C1QB	1	1	1	0	1	1	0	3
C1QC	2	0	1	1	1	1	1	3
ECM1	1	0	1	0	1	3	5	11
GSHR	0	0	0	0	1	1	0	6
HA10	0	0	1	0	1	2	1	7
HGFA	0	0	0	0	1	2	2	5

TABLE 3: (continued)

KV2A7	3	2	2	2	1	2	2	1
LAC1	1	1	1	0	1	1	1	3
IBP4	0	0	0	0	1	1	0	3
K1C17	0	1	8	14	1	0	0	0
K1C42	2	1	2	5	1	2	0	2
MUP1	16	13	1	14	1	1	1	0
MBL1	1	1	2	0	1	3	4	5
LCAT	0	0	2	0	1	1	1	5
PSA6	1	1	1	0	1	1	1	3
SAMP	1	1	0	1	1	1	2	5
SPRL1	0	0	0	0	1	3	3	4
QSOX1	0	0	0	0	1	1	2	7
THBG	0	0	0	0	1	0	1	3
1433Z	0	0	0	0	2	2	1	6
SAHH	0	1	0	0	2	3	2	1
ACE	3	1	2	0	2	4	4	4
ANXA3	0	0	0	0	2	3	0	0
APOC3	2	2	2	2	2	3	3	3
BTD	1	1	1	1	2	2	2	6
C4BPA	2	0	1	1	2	6	5	10
CO1A1	1	2	1	0	2	3	3	5
FCN1	0	0	1	0	2	3	3	5
G6PI	1	5	4	1	2	4	5	9
GSTM1 (+2)	0	0	0	0	2	4	3	3
HBA	9	5	5	5	2	4	4	4
IGJ	4	3	3	2	2	2	3	2
ITIH3	0	0	2	0	2	6	3	11
K1C16	0	0	0	3	2	1	0	1
K2C1	1	1	2	2	2	2	1	3
K2C73	3	2	2	3	2	2	2	1
LDHA	2	4	4	1	2	3	5	6
MDHC	0	1	0	0	2	2	1	3
PII6	3	0	0	0	2	2	1	2
PGM1	0	5	1	0	2	6	3	5
REG3B	0	0	0	0	2	3	2	2
SAA4	4	2	2	1	2	3	4	8

TABLE 3: (continued)

TFR1	0	0	0	0	2	4	1	5
AMY1	2	2	2	1	3	5	6	5
APOD	1	0	1	0	3	2	3	4
CO8G	2	1	3	1	3	4	5	7
CO9	2	0	1	0	3	4	4	7
KCRM	0	28	2	9	3	25	20	15
G3P	2	0	0	4	3	0	1	4
H2B1A (+11)	0	0	0	0	3	0	0	1
H4	1	0	0	0	3	0	0	4
KV3A1 (+1)	0	0	0	0	3	2	3	3
IBP6	1	0	0	0	3	3	3	0
K1C10	0	0	0	0	3	4	2	6
MUP2	3	3	3	2	3	3	3	2
RET4	0	0	1	0	3	2	3	5
SEPP1	1	1	1	0	3	1	2	6
SH3L3	1	1	0	1	3	1	1	2
ADIPO	4	2	4	2	4	5	4	5
A2AP	2	0	2	0	4	5	5	6
CAH2	6	4	4	2	4	5	4	4
CYC	0	0	1	0	4	3	3	2
FRIH	1	5	4	1	4	3	3	3
IL1AP	2	0	3	0	4	4	3	8
K22E	1	2	1	2	4	4	0	6
LUM	4	3	4	3	4	4	6	7
MUP3	3	2	4	2	4	8	5	7
PGK1	0	8	1	1	4	7	4	9
AMBP	4	2	2	2	4	4	5	7
WDR1	0	0	0	0	4	1	0	3
CLUS	5	3	7	2	5	6	5	14
CO8A	3	1	4	0	5	8	8	16
CO8B	4	0	6	0	5	6	10	14
GPX3	3	1	4	2	5	5	6	4
IGHA	7	3	6	1	5	6	6	4
IGKC	6	5	4	3	5	3	2	5
K2C6A	1	4	7	14	5	2	2	3
CSF1R	1	2	2	0	5	3	3	6

TABLE 3: (continued)

PPIA	2	2	2	1	5	6	4	5
IC1	5	1	5	2	5	6	5	8
ZPI	1	2	3	1	5	7	7	8
SPA3M	2	1	1	1	5	3	5	16
ANGT	4	4	5	3	6	7	7	8
CBPN	4	2	5	3	6	7	7	9
CPN2	2	3	6	3	6	10	7	11
CBPQ	1	3	3	2	6	7	4	5
FRIL1 (+1)	0	8	1	0	6	4	2	1
ITIH1	3	1	5	1	6	7	9	21
LIFR	11	3	10	2	6	10	9	17
LYZ2	0	0	0	0	6	1	3	2
THRB	4	0	3	1	6	10	9	15
KPYM	3	12	13	3	6	16	15	18
SPA3N	2	0	2	1	6	4	4	6
SODC	1	2	1	1	6	4	1	3
ZA2G	1	0	1	0	6	6	6	6
APOA2	6	4	5	6	7	7	7	4
CAH3	2	16	7	6	7	13	13	15
CATB	3	1	4	2	7	5	5	8
GUAD	2	2	0	0	7	0	3	8
ITIH4	0	0	0	0	7	0	1	12
VTNC	6	1	8	1	7	10	8	10
CD5L	4	3	5	2	8	7	5	8
FETUB	6	3	4	4	8	12	11	13
ALDOA	5	17	20	7	8	18	17	18
ALS	5	6	5	3	8	9	8	10
MUP20	5	5	5	3	8	4	3	4
PON1	4	3	5	2	8	7	8	11
TPIS	2	8	5	2	8	11	9	10
CBPB2	7	8	8	7	9	9	9	9
FA12	2	1	6	0	9	10	9	11
CO5	7	1	17	3	9	17	18	40
CFAD	8	7	7	7	9	7	7	6
ITIH2	5	0	3	2	9	18	18	16
K1C14	0	0	0	3	9	7	2	11

TABLE 3: (continued)

K2C5	0	1	2	6	9	12	0	15
PROP	8	4	8	0	9	11	12	10
PEDF	8	6	7	3	10	10	8	11
KLKB1	4	0	8	1	10	13	17	20
CFAI	9	2	7	4	11	13	15	18
HPT	11	8	11	9	11	11	9	21
ACTB (+1)	5	4	6	6	12	10	11	13
A1AT1	4	2	10	2	12	13	5	10
APOE	8	7	13	8	12	19	17	12
APOH	8	3	10	3	12	15	13	21
EGFR	9	2	9	2	12	14	13	17
PHLD	3	1	4	2	12	12	15	14
TTHY	14	15	12	13	12	11	11	7
FETUA	14	7	11	5	13	16	16	9
HRG	11	4	13	4	14	23	15	18
CBG	6	8	7	5	15	18	11	13
SPA3K	15	16	14	16	15	16	15	8
A1AG1	15	13	12	11	16	16	17	11
A1AT5	14	9	12	11	16	18	15	10
APOA4	18	4	18	7	16	18	18	21
KNG1	13	7	14	8	16	18	23	21
MUP6 (+2)	2	2	16	2	16	16	15	17
FIBG	16	11	16	11	17	28	22	21
ICA	17	7	13	5	17	26	25	23
A1AT4	17	39	37	38	19	19	17	25
CO4B	3	1	8	2	19	24	18	37
GELS	13	6	12	7	21	24	26	22
FIBA	28	17	21	17	22	32	30	24
IGHM	29	27	25	18	22	23	18	18
AMYP	6	9	7	2	22	14	14	16
PLMN	15	6	16	4	22	27	36	32
FIBB	25	13	20	9	26	30	32	27
ANT3	18	14	16	15	27	33	26	22
AFAM	22	23	23	15	30	30	28	26
APOA1	37	26	30	27	32	36	33	22
HEMO	40	33	38	27	32	35	37	28

TABLE 3: (continued)

EST1C	32	29	31	27	34	35	35	25
CFAB	26	13	24	13	35	30	33	32
CFAH	36	19	37	17	36	39	47	49
ALBU	8	8	27	11	38	36	37	31
A1AT2	45	17	14	15	43	45	44	10
FINC	30	18	30	8	48	51	43	74
VTDB	38	25	30	26	48	55	53	31
CERU	62	32	53	45	57	57	63	52
MUG1	56	37	50	36	63	70	73	65
PZP	0	0	0	0	81	106	106	74
CO3	79	52	91	64	110	114	108	97
TRFE	117	105	91	105	113	116	114	72

FIG. 7: Inflammatory effects due to plasma irradiation were very weak compared to cauterization. (a) IL-6 mRNA level in cecum was quantified by real-time PCR. IL-6 was induced at 9 hr after plasma irradiation. IL-6 level after plasma irradiation was not significantly different from the level induced by cauterization, but the trend of IL-6 in plasma irradiation appeared smaller than IL-6 in cauterization. (b) Lactoferrin (LTF) mRNA was strongly induced when cauterizing the cecum. Plasma irradiation showed little induction of LTF. ($N = 6$ in each experiment).

The results of inflammatory proteins identified by proteomics in ascites, such as lactoferrin, serum amyloid protein, inter- α -trypsin inhibitor, serine protease inhibitor, and apolipoprotein B released in peritoneal cavity, raised the possibility that are candidates associated with adhesion formation.⁴⁵ Quantitative mass spectroscopy⁴⁶ may help to reveal the mechanism underlying adhesion formation in the abdominal cavity.

Collectively, plasma irradiation affects less inflammatory response to abdominal tissues (Fig. 8).

FIG. 8: Schematic diagram of our results summarize plasma irradiation effects. Even though plasma irradiation of the tissue caused inflammation in the peritoneal cavity, few inflammatory cells were infiltrated in local tissue by plasma irradiation, which might be associated with whether adhesion occurs.

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