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Liver proteomics for therapeutic drug discovery: inhibition of the cyclophilin receptor CD147 attenuates sepsis-induced acute renal failure

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Abstract

Objective—Sepsis-induced multi-organ failure continues to have a high mortality. The liver is an organ central to the disease pathogenesis. The objective of this study was to identify the liver proteins that change in abundance with sepsis and, therefore, identify new drug targets.

Design—Proteomic discovery study and drug target validation

Setting—Research institute laboratory

Subjects—Three month old C57BL/6 mice

Interventions—We used a mouse model of sepsis based on cecal ligation and puncture (CLP) but with fluid and antibiotic resuscitation. Liver proteins that changed in abundance were identified by difference in-gel electrophoresis (DIGE). We compared liver proteins from 6 hr post-CLP to sham-operated mice ('early proteins') and 24 hr post-CLP with 6 hr post-CLP ('late proteins'). Proteins that changed in abundance were identified by tandem mass spectrometry. We then inhibited the receptor for one protein and determined the effect on sepsis-induced organ dysfunction.

Results—The liver proteins that changed in abundance after sepsis had a range of functions such as acute phase proteins, coagulation, ER stress, oxidative stress, apoptosis, mitochondrial proteins and nitric oxide metabolism. We found that cyclophilin increased in abundance after CLP. When the receptor for this protein, CD147, was inhibited sepsis-induced renal dysfunction was reduced. There was also a significant reduction in serum cytokine production when CD147 was inhibited.

Conclusion—By applying proteomics to a clinically relevant mouse model of sepsis we identified a number of novel proteins that changed in abundance. The inhibition of the receptor for one of these proteins, cyclophilin, attenuated sepsis-induced acute renal failure. The application of proteomics to sepsis research can facilitate the discovery of new therapeutic targets.

Introduction

Sepsis-induced multiple organ dysfunction has a high mortality and the liver plays a central pathophysiological role (1). The liver produces pro-coagulant factors, complement and acute phase proteins. It is also a major site for cytokine production and destruction and is critical for

bacterial clearance. Sepsis commonly injures the liver causing impaired regulation of inflammation and coagulation. Dysfunction also leads to changes in glucose metabolism, lactate clearance, and protein synthesis.

Proteomics is the large-scale study of the proteins in a cell, tissue or entire organism. Its potential utilization in biomedical research is enormous, for example to compare changes in protein levels when normal tissue is compared with disease (2). One established technique for comparative proteomics is based on labeling proteins from normal and diseased tissues with different fluorescent dyes (Cy3 and Cy5), mixing the proteins together then separating them by isoelectric point and molecular weight (difference in-gel electrophoresis - DIGE). As both Cy3 and Cy5 labeled samples are run on the same gel this reduces gel to gel variability (3). An internal standard labeled with Cy2 is included on all gels to facilitate comparison across gels (4). Tandem mass spectrometry can then be used to identify the proteins that have changed in abundance when Cy3 and Cy5 images are compared.

We have developed a mouse model of sepsis based on the commonly used cecal ligation and puncture (CLP) model but with fluid and antibiotic resuscitation to increase the model's clinical relevance (5,6). The mice develop multiple organ failure that resembles human sepsis. Because of the central role for the liver in sepsis we performed DIGE on this tissue with the objective of discovering new drug targets. A similar gel-based proteomic approach has been successful in the investigation of liver fibrosis and drug toxicity (7) (8). In our sepsis model it is the development of acute renal failure (ARF) that predicts mortality. We demonstrated this using a novel MRI technique and it is consistent with human studies that demonstrate ARF in a septic patient increases mortality (9) (10). Therefore we used renal function as the end-point to evaluate the efficacy of drug intervention.

Methods

Animals

Animal care followed National Institutes of Health (NIH) criteria for the care and use of laboratory animals. 3 month old C57BL/6 mice were obtained from Jackson Laboratories, Maine, USA. The mice had free access to water and chow (NIH-07 Rodent Chow) (Zeigler Bros., Gardners, PA, USA) before and after surgery.

Cecal ligation and puncture

The mice were anesthetized and polymicrobial sepsis was induced by performing CLP as previously described (5). All animals received a broad-spectrum antibiotic (imipenem/cilastatin; 14 mg/kg subcutaneously) in 1.5 ml of 3/4 normal saline at 6 hr after surgery and then every 12 hr. At the time of sacrifice, blood was collected by cardiac puncture. The liver was then harvested for histological evaluation, proteomic investigation and immunoblotting. The 10% formalin-fixed liver sections were stained with periodic acid-Schiff reagent (PAS). For proteomic investigation the liver tissue was homogenized in lysis buffer (see below). For immunoblotting the liver tissue was homogenized on ice in T-PER (Pierce, Rockford, IL, USA) with complete mini protease inhibitors (Roche, Indianapolis, IN, USA). Samples were aliquoted and stored at -80 °C prior to use.

In a subsequent target validation study, CLP was performed as above but either anti-CD147 antibody (25µg) or isotype control antibody was administered intraperitoneally at the time of surgery, 6 hr and 18 hr post-surgery. Blood was obtained and organs harvested 24 hr post-CLP surgery.

Two-dimensional difference in gel electrophoresis (DIGE)

We investigated sepsis-induced changes in the liver proteome by DIGE. Liver was homogenized in 7 M urea, 2 M thiourea, 4% CHAPS, 30 mM Tris, pH 8.8 and the protein concentration of the samples was determined using the 2-D Quant kit (Amersham, Bucks, UK). Liver protein (50µg) was then minimally labeled with cy5 dyes as per manufacturer's instructions (200 pmol per sample).

Two discovery experiments were performed to determine early (6 hr post-CLP vs 6 hr sham surgery) and late (24 hr post-CLP vs 6 hr post-CLP) changes in hepatic protein abundance. We used a dye-swap replication protocol for the discovery phase, and a parallel cutting gel for the identification phase (Table 1). The first 3 gels employ biological replication to increase statistical power, and the dye swap reduces labeling artifacts. The cutting gel contains a larger quantity of unlabeled protein to enhance the proteomic identification by MALDI-MS and tandem mass spectrometry.

Therefore, each discovery experiment used 6 mice, 3 per treatment group. The Cy2 labeled internal standard on each gel was created by pooling 8.3µg of liver protein from each of the 6 mice in an experiment (i.e. 50µg total). The cutting gel (gel 4) was created by pooling 100 µg of liver protein from each mouse.

Cy2-, Cy3- and Cy5-labeled samples were mixed together and rehydration buffer was added (7 M urea, 2 M thiourea, 4% CHAPS, 0.5% pharmalytes (Amersham), 40 mM dithiothreitol (DTT), and 0.002% bromophenol blue) to a final volume of 450µl. The samples were then loaded on an immobilized pH gradient strip (24 cm; pH 3–10) for isoelectric focusing according to the following protocol: 30 V for 14 hr (active rehydration), 500 V for 500 Vhr, 1,000 V for 1,000 Vhr, and 8,000 V for 66,667 Vhr. Strips were equilibrated for 15 min in a solution containing 50 mM Tris-HCl, pH 8.8, 6 M urea, 30% glycerol, 2% SDS, 0.002% bromophenol blue, and 65 mM DTT followed by a second 15-min equilibration with iodoacetamide (25 mg/ml) instead of DTT. Strips were briefly rinsed in 1X SDS-PAGE buffer and applied to a 12.5% polyacrylamide gel for electrophoresis at 5W/gel for 30 minutes followed by 17W/gel for 4 hrs.

Image acquisition and analysis

Images were scanned on a Typhoon 9400 variable mode imager (Amersham Biosciences) at an excitation wavelength of 520/40, Laser 488 (Blue2) for Cy2, 580/30 (laser 532 - green) for Cy3 and 670/30 (laser 633 - red) for Cy5 labeled samples. The laser power was chosen so that no saturated signal was obtained from any protein spot. The resolution was 100µm. Images were then processed with Progenesis Discovery software (Nonlinear Dynamics, Newcastle, UK). The software subtracts the background signal using a subtraction algorithm and quantifies spot intensity based on normalized spot volume. The normalization is ratiometric with Cy3 and Cy5 images being expressed as a ratio of the corresponding Cy2 internal standard protein spot. Statistical significance was determined by a two-sided student's t-test. Spot fluorescence intensities that were significantly increased or decreased with treatment were selected for identification. Selection was based on the change in intensity being statistically significant. No cut-off value for fold change was applied in order to maximize the yield of spots of interest.

After protein spots were designated to be picked, the gel with 600µg protein was fixed overnight in 30% ethanol and 3% phosphoric acid then stained in coomassie blue stain for 2 days. Then it was washed water for 1 day. Next, the gel was scanned at an excitation wavelength of 610/30 (laser 532) to visualize proteins present in the gel. CyDye- and coomassie blue - derived images were matched using Progenesis software from which a pick list was generated.

Spot picking and protein processing

The Ettan Spot Handling Workstation was used to subsequently cut out the selected protein spots from the 600µg protein gel, perform an in-gel tryptic digestion, extract the peptides from the gel, and transfer the digested proteins on a MALDI-TOF/Pro (Amersham Biosciences) sample slide. Peptides were analyzed using a 4700 Proteomics Analyzer (Applied Biosystems) tandem mass spectrometer.

Western blotting

Changes in protein abundance identified by DIGE were confirmed by western blot. The following antibodies were used: cyclophilin (USBiological, Swampscott, MA, USA), regucalcin (Cell Sciences, Canton, MA, USA), superoxide dismutase (USBiological, Swampscott, MA, USA) and catalase (Rockland, Gilbertsville, PA, USA). Western blot was performed as previously described (11). Equal protein loading for each lane was confirmed by immunoblotting for beta tubulin.

Statistical analysis

All data are expressed as mean $\pm$ SE. Differences between groups were analyzed for statistical significance by two sided student's T-test. A *P* value <0.05 was accepted as statistically significant.

Results

Liver injury occurred after CLP (figures 1 and 2). At both 6 and 24 hr after CLP there was an elevation in both serum aspartate transaminase (AST) and alanine transaminase (ALT) (figure 1). At 6 and 24 hr after CLP there was also injury on histological examination of the tissue (figure 2). The sham treated mouse liver was normal with even distribution of glycogen in hepatocytes and no evidence of lymphocyte infiltrate or Kupffer cell reactivity. At 6 hr post-CLP the liver showed loss of hepatocyte glycogen in watershed zones distant to vessels. Kupffer cells showed nuclear enlargement and were prominent with mild dilatation of sinuses. After 24 hr the liver was pale with loss of glycogen stores in all hepatocytes. Nuclei show altered chromatin rimming the nucleus. Kupffer cells were prominent and rare scattered neutrophils were present in the background.

We used DIGE to compare the liver proteome 6 hr after CLP surgery with 6 hr after sham surgery (experiment 1 – ‘early protein changes’). In a second experiment the liver proteome 6 hr after CLP surgery was compared with the liver proteome 24 hr after CLP surgery (experiment 2 – ‘late protein changes’). Figure 3 shows a typical 2D gel with examples of two proteins that changed in abundance. For experiment 1, 72 spots were selected of which 44 were correctly identified. For experiment 2, 62 spots were selected of which 52 were correctly identified. Identification was considered correct if the MS peptide mass fingerprinting score was above 90% and the measured MW and pI matched the expected values (table 1 and 2). The majority of the proteins identified had additional validation by amino acid sequencing with tandem mass spectrometry (table 1 and 2). The proteins that changed in abundance are shown graphically in figure 4 and figure 5.

Figure 6 compares changes in protein abundance as measured by DIGE and western blot. The changes detected by DIGE and by western blotting was always in the same direction, and of comparable magnitude.

Cyclophilin was one liver protein that changed in abundance following CLP – both by DIGE and western blot (figure 7). CD147 is a signaling receptor for extracellular cyclophilin and an antibody to this receptor was found to be anti-inflammatory in a mouse model of asthma

(12). When we inhibited this receptor with a specific blocking antibody there was a significant reduction in CLP – induced renal injury measured by both blood urea nitrogen and serum creatinine (figure 8). There was also a significant reduction in serum TNF- α , IL-6 and IL-10 concentration 24 hr post-CLP when anti CD147 antibody was compared to vehicle (figure 9) (serum TNF- α 24 hr post-CLP: sham antibody 225 ± 40 pg/ml; anti-CD147 antibody 105 ± 25 pg/ml $n=8$ $p=0.03$. Serum IL-6 24 hr post-CLP: sham antibody 82 ± 14 ng/ml; anti-CD147 antibody 16 ± 7 ng/ml $n=8$ $p=0.01$. Serum IL-10 24 hr post-CLP: sham antibody 1370 ± 146 pg/ml; anti-CD147 antibody 667 ± 265 pg/ml $n=8$ $p=0.03$). Serum amylase concentration was also reduced by the antibody but there was no significant difference in serum transaminase concentration (figure 10).

Discussion

In this study we used the CLP model of polymicrobial sepsis to induce liver injury and used DIGE to explore changes in the liver proteome. These tissue changes were validated by western blot. Recent studies have demonstrated that extracellular cyclophilin is proinflammatory via the CD147 receptor and that anti-CD147 antibodies are anti-inflammatory (12,13,14,15,16). Administration of anti-CD147 antibodies significantly reduced sepsis-induced acute renal failure and reduced both pro and anti inflammatory cytokine production.

The liver proteins that changed in abundance with sepsis had a range of functions such as acute phase proteins, coagulation, ER stress, oxidative stress, apoptosis, mitochondrial proteins and nitric oxide metabolism. Six hr after CLP the protein with the largest increase in abundance was calgranulin B. This protein is a marker of neutrophils and has been demonstrated to be elevated in liver after administration of endotoxin (LPS) (17). Fibrinogen beta precursor had the largest decrease 6 hr post-CLP. Changes in coagulation are well-described in sepsis (18). After 24 hr the proteins with the largest increase relate to amyloid formation. Among the proteins with the largest fold decrease 24 hr after CLP is carbamoyl phosphate synthetase. Fragmentation of this protein occurs in the liver after LPS administration and fragments can be detected in the serum (19). We will discuss the possible significance of a few of the results then focus on cyclophilin in the pathophysiology of sepsis.

Catalase protein levels changed in abundance consistent with the presence of oxidative stress in the liver after CLP (as described previously (20)). The increase in the enzyme cysteine-sulfinate decarboxylase may also reflect oxidative stress. This enzyme is important in taurine synthesis and taurine plays an important role in the antioxidant response to inflammation (21). For example, after bacterial injection into rats taurine synthesis increases in the liver as an antioxidant mechanism (22). Glutathione is another important antioxidant and glutathione-S-transferase was increased in the liver after CLP consistent with previous reports (23) (24).

The decrease in AdoMet synthetase post – CLP is a novel finding which is interesting as the product of this enzyme, S-adenosylmethionine, has anti-inflammatory properties such as inhibition of cytokine production by kupffer cells after LPS exposure *in vitro* and reduction of mortality after LPS injection *in vivo* (25) (26) (27).

Regucalcin (RC) (Senescence marker protein-30) is a calcium-regulated aging marker whose gene transcription is regulated by LPS (28) and has been found to be a biomarker of carbon tetrachloride-induced liver injury (29). By DIGE and western blot we found the liver abundance was increased early after CLP which is interesting as SMP-30 has been demonstrated to protect hepatocytes from TNF α - and Fas- induced hepatocyte apoptosis *in vivo* (30).

NG,NG-dimethylarginine dimethylaminohydrolase 1 (DDAH) hydrolyzes ADMA which acts as an inhibitor of nitric oxide synthesis and therefore this enzyme has a regulatory role in nitric oxide generation (31,32). Nitric oxide may be toxic or protective in the liver in sepsis (33) and

changes in DDAH level in sepsis may reflect low liver blood flow as has been demonstrated in the dog heart by 2 dimensional electrophoresis (34).

Cyclophilin A is an abundant intracellular protein and is the target for the immunosuppressive drug cyclosporine (reviewed by Yurchenko et al.(16)). Cyclophilin is released by cells in response to inflammation and elevated serum levels have been demonstrated in humans with severe sepsis (35). Cyclophilin has pro-inflammatory actions, for example as a chemotactic agent for inflammatory cells (13) and an activator of endothelial cells (14,15). Therefore, we choose this protein as a potential therapeutic target. Yeast two hybrid studies identified CD147 as an extracellular receptor for cyclophilin and further studies demonstrated that this receptor mediates the pro-inflammatory actions of cyclophilin (16). In our study when we inhibited CD147 we significantly reduced sepsis-induced renal injury. Our previous studies have demonstrated that renal dysfunction predicts mortality following CLP (10), which is consistent with human studies that demonstrate acute renal failure (ARF) in a septic patient increases mortality (9). Thus far, in humans, few therapeutic interventions have successfully reduced the incidence of sepsis-induced ARF. Therefore drugs targeting inhibition of cyclophilin offer a potential much needed novel therapy. We also found reduced serum levels of TNF- α , IL-6 and IL-10 cytokines after anti CD147 antibody administration. This is consistent with the pro-inflammatory action of cyclophilin in other models (12). Serum amylase concentration is significantly elevated after CLP and this elevation was significantly reduced by administration of anti-CD147 antibody. This may reflect a reduction in pancreatic injury or be a result of the reduction in renal injury due to amylase being excreted via the kidneys (36).

There are still significant questions regarding the role of cyclophilin in sepsis. The critical site of cyclophilin release is unknown. We demonstrated an increase in the liver, a central organ in sepsis pathophysiology, but whether other organs play a more or less significant role is unknown. It is interesting that administration of anti-CD147 antibody did not significantly reduce sepsis-induced liver injury as measured by serum AST and ALT levels. This may be another indication that different pathways mediate injury in the liver as compared with the kidney (6) or may represent a type 2 statistical error secondary to the insensitivity of transaminase enzymes at detecting liver dysfunction. However, using mice lacking the protein MyD88 we previously demonstrated that the degree of liver injury (as measured by AST and ALT) does not correspond with sepsis mortality but renal injury closely predicted survival (6,10). Therefore, we believe it is an important finding that anti-CD147 prevented ARF even in the absence of a significant effect on liver injury.

As a proteomic discovery technique DIGE has limitations. A number of proteins whose abundance changed following sepsis will not have been identified. Such false negatives will occur with gel-based proteomics because of multiple reasons, such as difficulty in detecting low abundance proteins or the inability to solubilize membrane proteins. As we used correct pI and molecular weight as criteria for protein identification we will miss a number of post-translational modifications. Even high abundance, soluble proteins may not be amenable to identification by mass spectrometry because of technical limitations. Approaches that are more likely to complement DIGE and result in greater coverage of the proteome, such as separation by liquid chromatography prior to identification by mass spectrometry or protein antibody arrays will further increase our understanding of sepsis (3). Also advances in mass spectrometry will increase the identification rate of interesting proteins.

Conclusion

In this study we have used DIGE to characterize sepsis-induced changes in the liver proteome. By inhibiting the action of one protein, cyclophilin, we were able to reduce sepsis-induced

renal injury. The application of proteomics to the investigation of sepsis has the potential to yield further valuable insights.

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Figure 1. Serum transaminase levels 6 and 24 hr after CLP. A) Serum aspartate transaminase (AST) concentration 6 hr after sham surgery and 6 and 24 hr after CLP surgery n=3 per group. B) Serum alanine transaminase (ALT) concentration 6 hr after sham surgery and 6 and 24 hr after CLP surgery. (n=3 per group).

Figure 2. Histological liver injury after CLP. Typical histology is presented 6 hr post sham surgery, 6 hr post CLP and 24 hr post CLP. The upper panels are 10× and lower panels are 40× original magnification. PAS stained sections (Bar = 50μM)

Figure 3.

Typical two-dimensional electrophoresis gel. Proteins increased in abundance 6 hr after CLP are red. Proteins decreased in abundance 6 hr after CLP are green. An example of proteins which increased (A) and decreased (B) in abundance are shown.

Figure 4.

Liver proteins identified by DIGE which increased or decreased in abundance 6 hr after CLP (compared with sham). The fold change is expressed as a ratio of protein abundance 6 hr post-CLP / protein abundance 6 hr post-sham surgery. Error bars represent S.E.M.

Figure 5.

Liver proteins identified by DIGE which increased or decreased in abundance 24 hr after CLP (compared with 6 hrs post-CLP). The fold change is expressed as a ratio of protein abundance 24 hr post-CLP / protein abundance 6 hr post-CLP. Error bars represent S.E.M.

Figure 6.
The sepsis-induced fold change in liver protein abundance as measured by western blot and DIGE.

Figure 7.
Western blot for cyclophilin in liver tissue after sham and 24 hr post-CLP surgery.

Figure 8.

Anti CD147 antibody significantly reduces renal injury 24 hr post-CLP. A) Renal injury measured by blood urea nitrogen. B) Renal injury measured by HPLC serum creatinine (n=18-20 per group; * p<0.05).

Figure 9.

Anti CD147 antibody significantly reduces serum cytokine levels 24 hr post-CLP. Serum TNF- α (A), IL-6 (B), and IL-10 (C) concentrations after vehicle or anti CD147 antibody treatment at 0, 6, and 18 hrs post surgery (n= 5-9 per group; * p<0.05).

Figure 10.

Anti CD147 antibody significantly reduces pancreatic injury but does not significantly reduce liver injury 24 hr post-CLP. A) Pancreatic injury measured by serum amylase concentration. B) Liver injury measured by serum aspartate transaminase C) Liver injury measured by serum alanine transaminase (n=13-20 per group; * $p<0.05$).

Table 1

Experimental design for DIGE study

GEL	Condition A	Condition B	Internal standard
GEL1	CY3 (Mouse 1)	CY5 (Mouse 2)	CY2
GEL2	CY5 (Mouse 3)	CY3 (Mouse 4)	CY2
GEL3	CY3 (Mouse 5)	CY5 (Mouse 6)	CY2
GEL4	600 µg of unlabeled internal standard		

Liver proteins that changed abundance when mice at 6 hr post-CLP were compared with sham-operated mice

Proteins were identified by MALDI-TOF-TOF. The change is expressed as fold increase or decrease of protein abundance at 6 hr post-CLP vs. sham surgery. A positive percentage indicates an increase at 6 hr of sepsis; a negative indicates a decrease at 6 hr sepsis. The measured molecular weight (MW) and measured isoelectric point (pI) were determined from the 2D gel. Also shown are the predicted MW and pI. The identifications were made by peptide mass fingerprinting and tandem mass spectrometry for most proteins and the scores are shown as a percentage.

Table 1

CHANGE	P=	Protein Name	Swissprot No	Protein MW	Measured MW	Protein pI	Measured pI	Peptide Count	Peptide mass fingerprinting score	MS/MS score
10.3	0.024	(P31725) 14) (P14) (Leukocyte L1 complex heavy chain).	S109_MOUSE	13	15	6.7	7	6	100	100
7.1	0.004	(P16015) Carbonic anhydrase 3	CAH3_MOUSE	30	20	7.0	7	14	100	99.94
6.5	0.020	(P07724) Serum albumin precursor.	ALBU_MOUSE	71	80	5.8	6	12	100	91.767
5.2	0.030	(Q9D0F9) Phosphoglucomutase (EC 5.4.2.2)	PGMU_MOUSE	62	70	6.3	7	8	99.869	0
5.1	0.001	(Glucose phosphomutase) (PGM).								
		(Q9DBF1) Aldehyde dehydrogenase family 7 member A1 (EC 1.2.1.3) (Antiquitin 1).	D7A1_MOUSE	56	50	6.0	6	11	100	91.449
4.6	0.004	(Q8K183) Pyridoxal kinase (EC 2.7.1.35)	PDXK_MOUSE	35	30	5.9	6	9	100	99.047
		(Pyridoxine kinase).								
3.9	0.007	(P10649) Glutathione S-transferase GT8.7)	GTM1_MOUSE	26	30	8.1	8	11	100	99.879
		(pmGT10) (GST 1-1).								
3.3	0.023	(P10649) Glutathione S-transferase GT8.7)	GTM1_MOUSE	26	25	8.1	8	21	100	100
		(pmGT10) (GST 1-1).								
3.2	0.030	(Q99KB8) Hydroxyacylglutathione hydrolase	GLO2_MOUSE	29	35	6.5	7	7	99.956	92.275
2.8	0.050	(Q64442) Sorbitol dehydrogenase.	DHSO_MOUSE	41	40	6.6	7	13	100	99.983
2.8	0.030	(Q9DBE0) decarboxylase) (Cysteine-sulfinate decarboxylase).	CSD_MOUSE	56	50	6.2	7	19	100	99.708
2.5	0.005	(O08557) (DDAH1) (DDAH-1).	DDH1_RAT	32	40	5.8	5	4	92.777	0
2.5	0.020	(P12710) Fatty acid-binding protein, liver (L-FABP)	FABL_MOUSE	14	15	8.6	8	10	100	100
2.2	0.020	(P07724) Serum albumin precursor.	ALBU_MOUSE	71	70	5.8	6	22	100	99.855
2.2	0.040	(P17742) Cyclophilin A) (Cyclosporin A-binding protein) (SPI8).	PPIA_MOUSE	18	20	7.9	7	3	99.913	99.929
2.2	0.005	(P52760) Ribonuclease UK114 (EC 3.1.-.-) (Heat-responsive protein 12).	UK14_MOUSE	14	15	8.7	8	9	100	100
2.2	0.038	(P26772) (CPN10).	CHI0_RAT	11	10	8.9	7	6	100	99.991
2.1	0.020	(P47738) AHD-M1 (ALDH1) (ALDH-E2).	DHAM_MOUSE	57	50	7.5	6	18	100	99.973
2.1	0.002	(P50247) (Liver copper binding protein) (CUBP).	SAHH_MOUSE	48	40	6.1	6	18	100	99.459
2.1	0.034	(P97532) 3-mercaptopyruvate sulfurtransferase (EC 2.8.1.2) (MST).	THTM_RAT	33	40	5.9	6	7	100	99.703
2.0	0.058	(P10860) Glutamate dehydrogenase, mitochondrial precursor (EC 1.4.1.3) (GDH).	DHE3_RAT	62	55	8.1	7	10	100	64.692
2.0	0.020	(Q91XD4) (EC 4.3.1.4) (Formiminotetrahydrofolate cyclodeaminase)].	FTCD_MOUSE	60	60	5.8	5	25	100	99.616
1.9	0.030	(P24270) Catalase (EC 1.11.1.6).	CATA_MOUSE	60	50	7.7	8	15	100	99.963
1.9	0.006	(P55264) Adenosine kinase	ADK_MOUSE	31	40	7.2	5	10	100	71.946
1.9	0.010	(P01942) Hemoglobin alpha chain.	HBA_MOUSE	15	15	8.1	7	8	100	100
1.8	0.050	(P97429) Annexin A4 (Annexin IV).	ANX4_MOUSE	36	30	5.4	5	13	100	87.204
1.8	0.180	(P30115) Glutathione S-transferase Yc (EC 2.5.1.18) (GST class-alpha) (Ya3).	GTC_MOUSE	25	30	8.8	9	17	100	100
1.6	0.060	(P40956) Thioether S-methyltransferase (EC 2.1.1.96) (TEM1).	TEMT_MOUSE	30	40	6.0	6	8	100	99.397

CHANGE	P=	Protein Name	Swissprot No	Protein MW	Measured MW	Protein PI	Measured PI	Peptide Count	Peptide mass fingerprinting score	MS/MS score
1.6	0.060	(O88844) (Oxalysuccinate decarboxylase) (IDH) (NADP+-specific ICDH) (IDP), (P29758) (Omithine--oxo-acid aminotransferase).	IDHC_MOUSE	47	40	6.5	6	17	100	91.773
1.6	0.002	(Q64374) Senescence marker protein-30 (SMP-30) (Regucalcin) (RC), (P09103) (p55) (Erp59), (P68372) Tubulin beta-? chain, (P62880) (Transducin beta chain 2) (G protein beta 2 subunit).	OAT_MOUSE SM30_MOUSE PDL_MOUSE TBBX_MOUSE GBB2_MOUSE	49 34 58 50 38	40 20 50 45 40	6.2 5.2 4.8 4.8 5.6	6 5 5 5 5	15 16 24 21 8	100 100 100 100 99.968	95.715 99.997 100 99.522
-1.4	0.010	(P14206) 40S ribosomal protein SA (p40) (34/67 kDa laminin receptor).	RSP4_MOUSE	33	30	4.7	5	8	100	99.999
-1.5	0.028	(P54869) Hydroxymethylglutaryl-CoA synthase.	HMCN_MOUSE	54	50	8.0	7	11	100	99.998
-1.8	0.017	(P07758) (Alpha-1 protease inhibitor 1) (Alpha-1-antitrypsin) (AAT).	A1T1_MOUSE	46	50	5.4	5	9	99.994	
-1.9	0.020	(P06151) L-lactate dehydrogenase A chain, precursor	LDHA_MOUSE GR78_RAT	37 72	30 70	7.8 5.1	8 5	13 26	100 100	96.922 100
-2.9	0.008	(P14480) Fibrinogen beta chain precursor [Contains: Fibrinopeptide B].	FIBB_RAT	55	60	7.9	7	8	99.937	99.871
-3.0	0.060	(P13444) (Methionine adenosyltransferase) (AdoMet synthetase) (MAT-4/III).	METL_RAT	44	50	5.6	5	15	100	99.999
-3.3	0.059	(P14480) Fibrinogen beta chain precursor [Contains: Fibrinopeptide B].	FIBB_RAT	55	50	7.9	7	9	99.997	99.859

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Table 2

Liver proteins that changed abundance when mice at 24 hr post-CLP were compared with mice 6 hr post-CLP

The change is expressed as fold increase or decrease protein abundance of 24 hr vs. 6 hr post-CLP. A positive percentage indicates protein abundance is increasing; a negative indicates protein abundance is decreasing. Data are displayed as in Table 1.

CHANGE	p=	Protein Name	Swissprot No	Protein MW	Measured MW	Protein PI	Measured PI	Peptide Count	Peptide mass fingerprinting score
7.4	<0.001	(P05367) Amyloid fibril protein AA.	SAA2_MOUSE	14	10	6.4	6.0	9	100.0
6.3	<0.001	(P05366) Serum amyloid A-1 protein precursor.	SAA1_MOUSE	14	15	6.5	6.5	8	100.0
3.5	<0.001	(P14480) Fibrinogen beta chain precursor [Contains: Fibrinopeptide B].	FIBB_RAT	55	60	7.9	6.0	25	100.0
2.4	<0.001	(Q922C0) Serotransferrin precursor.	TRFE_MOUSE	79	100	6.9	7.5	54	100.0
2.2	<0.001	(P02680) Fibrinogen gamma chain precursor.	FIBG_RAT	51	50	5.4	5.0	16	99.6
2.0	<0.001	(P20029) 78 kDa glucose-regulated protein precursor (BiP).	GRP78_MOUSE	72	75	5.1	5.0	39	100.0
1.9	<0.001	(Q64152) Transcription factor BTF3 (RNA polymerase B transcription factor 3).	BTF3_MOUSE	22	20	9.4	7.5	8	100.0
1.9	<0.001	(P01942) Hemoglobin alpha chain.	HBA_MOUSE	15	15	8.1	8.0	11	100.0
1.8	<0.001	(P62716) isoform (EC 3.1.3.16) (P2A-beta).	P2AB_RAT	36	35	5.2	4.7	17	100.0
1.6	0.003	(P08113) Tumor rejection antigen gp96.	ENPL_MOUSE	93	140	4.7	4.5	62	100.0
1.6	<0.001	(Q9QXD6)FBPase	FI6P_MOUSE	37	40	6.2	6.5	27	100.0
1.5	<0.001	(P07724) Serum albumin precursor.	ALBU_MOUSE	71	70	5.8	5.5	49	100.0
1.4	0.002	(Q91X91) QPR Tase.	NADC_MOUSE	32	30	6.2	6.5	17	100.0
1.4	0.010	(O70435) Proteasome subunit K.	PSA3_MOUSE	28	30	5.3	6.0	15	99.6
1.4	<0.001	(P63324) 40S ribosomal protein S12.	RS12_RAT	15	15	7.0	7.0	10	100.0
1.3	0.003	(P55264) (Fragment).	ADK_MOUSE	31	40	7.2	6.0	12	100.0
1.3	<0.001	(P14152) Malate dehydrogenase, cytoplasmic (EC 1.1.1.37).	MDHC_MOUSE	36	35	6.2	6.0	20	100.0
1.3	0.001	(P23492) PNP.	PNPH_MOUSE	33	30	5.8	5.5	20	100.0
1.2	0.008	(P63260) Actin, cytoplasmic 2 (Gamma-actin).	ACTG_MOUSE	42	40	5.3	5.0	27	100.0
1.2	0.003	(Q64433) CPN10.	CH10_MOUSE	11	10	8.2	7.0	8	100.0
-1.1	0.020	(O08709) (EC 1.11.1.7) (NSGPx).	PRDX6_MOUSE	25	30	5.7	6.0	21	100.0
-1.2	0.010	(Q63880) Esterase 31.	EST31_MOUSE	62	65	5.9	6.0	23	100.0
-1.2	0.003	(P97823) (LysoPLA I) (Lysophospholipase I).	LYPAL_MOUSE	25	30	6.1	6.0	9	100.0
-1.3	<0.001	(P14152) Malate dehydrogenase, cytoplasmic (EC 1.1.1.37).	MDHC_MOUSE	36	35	6.2	5.8	16	100.0
-1.3	<0.001	(P40936) Thioether S-methyltransferase (EC 2.1.1.96) (TEM1).	TEMT_MOUSE	30	30	6.0	6.0	19	100.0
-1.4	0.010	(P26043) Radixin (ESP10).	RADL_MOUSE	69	90	5.9	6.0	43	100.0
-1.4	0.009	(P10518) Porphobilinogen synthase (ALADH).	HEM2_MOUSE	36	40	6.3	6.5	16	99.8
-1.4	0.050	(P08226) Apolipoprotein E precursor (Apo-E).	APOE_MOUSE	36	30	5.6	5.0	23	100.0
-1.5	0.006	(O08807) AOE372.	PRDX4_MOUSE	31	30	6.7	5.5	16	100.0
-1.5	<0.001	(P117742) (Cyclophilin A) (Cyclosporin A-binding protein) (SP18).	PPIA_MOUSE	18	20	7.9	8.0	12	100.0

CHANGE	p=	Protein Name	Swissprot No	Protein MW	Measured MW	Protein PI	Measured PI	Peptide Count	Peptide mass fingerprinting score
-1.5	<0.001	(P56395) Cytochrome b5.	CYB5_MOUSE	15	15	5.0	4.5	10	100.0
-1.6	<0.001	(Q64374) Senescence marker protein-30 (SMP-30) (Regucalcin) (RC).	SM30_MOUSE	34	30	5.2	4.5	26	100.0
-1.6	0.055	(P40936) Thioether S-methyltransferase (EC 2.1.1.96) (TEM1).	TEM1_MOUSE	30	30	6.0	6.0	13	100.0
-1.6	0.030	(Q9CQ60) 6-phosphogluconolactonase (EC 3.1.1.31) (6PGL).	6PGL_MOUSE	27	30	5.6	5.0	6	100.0
-1.7	0.004	(Q8CAQ8) Mitochondrial inner membrane protein (Mitofilin).	IMMT_MOUSE	84	85	6.2	6.0	39	100.0
-1.7	<0.001	(P16015) Carbonic anhydrase 3	CAH3_MOUSE	30	30	7.0	7.0	21	100.0
-1.7	0.002	(P24472) GST A4-4.	GSTA4_MOUSE	26	30	6.8	6.5	15	100.0
-1.8	<0.001	(P40936) Thioether S-methyltransferase (EC 2.1.1.96) (TEM1).	TEM1_MOUSE	30	30	6.0	5.5	16	100.0
-1.9	0.020	(P30416) FK506-binding protein 4	FKBP4_MOUSE	52	55	5.5	5.0	28	100.0
-1.9	<0.001	(P70349) Histidine triad nucleotide-binding protein 1	HINT1_MOUSE	14	15	6.4	6.8	7	100.0
-2.0	0.040	(P07756) (EC 6.3.4.16) (Carbamoyl-phosphate synthetase I) (CPSase I).	CPSM_RAT	166	160	6.3	6.5	67	100.0
-2.0	0.006	(P07756) (EC 6.3.4.16) (Carbamoyl-phosphate synthetase I) (CPSase I).	CPSM_RAT	166	100	6.3	6.5	49	100.0
-2.0	0.020	(Q9R1P3) Proteasome subunit beta type 2	PSB2_MOUSE	23	25	6.5	6.0	6	94.5
-2.1	0.010	(P08003) (ERp72).	PDIA4_MOUSE	72	80	5.1	5.0	18	100.0
-2.1	0.002	(P16331) monooxygenase	PH4H_MOUSE	52	45	6.0	5.5	34	100.0
-2.2	<0.001	(P07756) (EC 6.3.4.16) (Carbamoyl-phosphate synthetase I) (CPSase I).	CPSM_RAT	166	160	6.3	6.0	46	100.0
-2.3	<0.001	(P07756) (EC 6.3.4.16) (Carbamoyl-phosphate synthetase I) (CPSase I).	CPSM_RAT	166	90	6.3	5.5	28	100.0
-2.4	0.003	(P08228) Superoxide dismutase [Cu-Zn] (EC 1.15.1.1).	SODC_MOUSE	16	20	6.0	6.0	15	100.0
-2.6	0.015	(P07756) (EC 6.3.4.16) (Carbamoyl-phosphate synthetase I) (CPSase I).	CPSM_RAT	166	160	6.3	6.5	49	100.0
-2.6	0.007	(Q9QXE0) 2-hydroxyphenylanyl-CoA lyase (EC 4.1.-.-) (2-HPCL).	HPCL_MOUSE	65	65	5.9	6.0	18	99.4
-2.6	0.020	(P17182) neutral enolase(NNE) (Enolase 1).	ENOA_MOUSE	47	50	6.4	6.0	12	98.0
-2.9	0.010	(P08228) Superoxide dismutase [Cu-Zn] (EC 1.15.1.1).	SODC_MOUSE	16	20	6.0	6.0	7	100.0