Inflammation, Vol. 42, No. 6, December 2019 (© 2019)
DOI: 10.1007/s10753-019-01063-5

ORIGINAL ARTICLE

Investigation of Extracellular Matrix Protein Expression Dynamics Using Murine Models of Systemic Inflammation

Chandra Bhan,[1] Shiba Prasad Dash,[1] Pankaj Dipankar,[1] Puneet Kumar,[1] Papiya Chakraborty,[1] and Pranita P. Sarangi[1,2]

Abstract— Extracellular matrix (ECM) proteins form the structural support for migration of leukocytes and provide multiple signals to assist in their functions during inflammatory conditions. Presence of pro-inflammatory mediators in the tissues results in the remodelling of matrices which could modify the functions of extravasated leukocytes. Previous reports have shown changes in the expression of ECM proteins during local inflammatory responses. In this study, we have investigated the time- and tissue-specific expression profile of key ECM proteins in systemic inflammation using lipopolysaccharide (LPS)-induced endotoxemia and cecal ligation and puncture (CLP) mouse models. The results show that compared to naïve tissues, within 12 h following CLP surgery, a 20–30-fold increase was observed in the expression of collagen-IV (Col-IV) transcripts in the mesentery tissues with a 2.4-fold increase in the protein by 24 h. However, Western blot band intensities indicated that vimentin and fibrinogen were remarkably expressed in more quantity compared to Col-IV. Secondly, in CLP group of mice, fibrinogen showed 6–40-fold increase in mRNA level in various tissues with about 2-fold increase in the protein level compared to respective naïve tissues. Similar studies in the LPS-injected mice showed up to 2–3 fold increase in the expression of Col-IV, fibrinogen and vimentin at protein level in the lungs. In such animals, although similar pattern was observed for fibrinogen in kidney and liver tissues, the mesentery showed prominent changes in Col-IV and vimentin mRNA compared to CLP. Further, bioinformatics analysis showed multiple pathways which could be associated with

Chandra Bhan and Shiba Prasad Dash contributed equally to this work.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10753-019-01063-5) contains supplementary material, which is available to authorized users.

[1] Department of Biotechnology, Indian Institute of Technology, Roorkee, Uttarakhand 247667, India
[2] To whom correspondence should be addressed at Department of Biotechnology, Indian Institute of Technology, Roorkee, Uttarakhand 247667, India. E-mail: psarafbt@iitr.ac.in

vimentin, Col-IV and fibrinogen under inflammatory conditions both in human and mouse. The current study will help in better understanding of possible signalling from ECM proteins in inflammatory microenvironment and may contribute in development of cell adhesion-based therapeutics.

KEY WORDS: extra cellular matrix; inflammation; sepsis.

INTRODUCTION

Extracellular matrix (ECM) is a key modulator of inflammation. It not only provides the structural support for migration of immune cells but also acts as a repository of extracellular signalling molecules [1]. Following extravasation, the immune cells interact with a variety of matrix components such as collagen, fibronectin and laminin which could modify their functional properties [2]. Similarly, secretion of inflammatory mediators such as cytokines, MMPs and protease from inflammatory cells influence the expression and distribution of ECM in the inflamed tissue contributing to different cellular phenotypes [3]. Thus, ECM remodelling is a prominent pathological feature in many inflammatory diseases [2, 4].

Sepsis is defined as a dysregulated systemic inflammatory response to an uncontrolled infection and is a leading cause of death in adult intensive care units around the world [5]. Previous histopathological analysis of visceral tissue from septic patients has demonstrated that the dysregulated microenvironment could lead to hyperactivation, altered migration and activation of innate immune cells such as neutrophils and macrophages [6, 7]. Furthermore, excessive production of pro-inflammatory mediators, matrix metalloproteinases (MMPs) and reactive oxygen species by innate immune cells could lead to further tissue damage leading to multi organ failure [5]. To date, many studies have focused on the molecular mechanisms of altered trafficking of immune cells and disease-specific local ECM remodelling. For example, previous studies in local inflammatory conditions such as arthritis, brain injury, lung injury *etc.* have shown changes in the expression of multiple ECM proteins such as Col-IV, fibrinogen, vimentin, fibronectin and laminin [2, 8, 9].

The current study is based on the hypothesis that systemic inflammation could differentially alter the expression and distribution of ECM proteins in both local and distant visceral tissues. Our results from two murine models of systemic inflammation which involve different inflammatory events showed tissue-specific differences in the expression of Col-IV, fibrinogen and vimentin at mRNA and protein level with few unique pattern in both models. The changes in the expression of these proteins were observed earlier at the site of inflammation compared to the distant visceral organs. These changes could be associated with sequence of inflammatory changes taking place in various tissues at a given time. Further, bioinformatics analysis showed multiple pathways which could be associated with specific ECM proteins under inflammatory conditions both in human and mouse. The results from this study will open a new avenue towards understanding the complex pathophysiology of sepsis and may lead to development of cell adhesion-based therapeutics.

METHODS

Sepsis Mouse Model. Cecal ligation and puncture (CLP) and endotoxemia were conducted on C57BL/6j in accordance with protocol approved by the Animal Ethics Committee at Indian Institute of Technology Roorkee, India. For both procedures, protocols were followed as described earlier by Sarangi et al. [10]. Six- to eight-week C57BL/6j male mice were purchased from National Animal Resource Facility for Bio-Medical Research, Hyderabad, India. Endotoxemia was performed by injecting 36.7 mg/kbw of lipopolysaccharide (LPS) (*Eschericia coli* 026:B6, Sigma Aldrich, St. Louis, MO) in 6–8-week mice (22–25 g) intraperitoneally as per the titration in the animals to achieve 70–80% mortality. CLP surgery was performed in mice anaesthetized with ketamine and xylazine. Following midline incision, caecum was taken out and ligated with silk suture. Following ligation, the caecum was punctured through and through using a 21 gauge needle. Following wound closure with silk suture, 1% lignocaine was applied to the surgery site. One millilitre of dextrose normal saline (DNS) was injected subcutaneously to resuscitate the animals following CLP surgery. For both the models, access to soft pellets and water was made available throughout the experimentation period.

Real-Time PCR. Total RNA was extracted from the tissues using TRIzol Reagent (Life technologies, Carlsbad, CA), and reverse transcription of the extracted RNA was performed using Accuscript High Fidelity 1st Strand

Table 1. List of Primers

S. No.	Primer (gene) name	Primer sequence
1.	HPRT-F	GTTAAGCAGTACAGCCCCAAA
2.	HPRT-R	AGGGCATATCCAACAACAAACTT
3.	Collagen-Iα1-F	ACCTCAAGATGTGCCACTCT
4.	Collagen-Iα1-R	TGACCTGTCTCCATGTTGCA
5.	Collagen-IVα1-F	GGGGCGAGAACTCCATAAGA
6.	Collagen-IVα1-R	GGTTGGACAGCACTCACATC
7.	Fibronectin-F	CCGTTCCAGGAGAGTTCTGA
8.	Fibronectin-R	TCGCACTGGTAGAAGTTCCA
9.	Fibrinogen α-F	CAGGGTCAAAGGCAGAAAAC
10.	Fibrinogen α-R	CAGGGTTCCGATTTCCATCA
11.	Laminin-IVα-F	CCCCTGCCTTTTGATGTTCA
12.	Laminin-IVα-R	GCGTGGTCTCTTCTCTCTCT

cDNA Synthesis Kit (Agilent Technologies, Santa Clara, CA) according to the manufacturer's instructions. Real-time PCR was performed using Power SYBR Green PCR master mix and Power UP SYBR Green master mix (Applied Biosystems, Foster City, CA) with Primers listed in the Table 1. Samples were normalized to the endogenous control HPRT, and relative gene expression was measured by $2^{-\Delta\Delta CT}$ method.

Protein Isolation and Western Blotting. Total protein was isolated from the animal tissues using T-PER™ Tissue Protein Extraction Reagent (Thermo Fisher Scientific, Walthem, MA). An equal amount of protein was loaded for SDS-PAGE. The separated proteins were transferred onto BioTrace NT nitrocellulose membrane (PALL Corporation, New York) and blocked with 5% BSA or 5% dried, fat-free milk in PBS-0.05% Tween-20 (PBST) for 1–2 h at room temperature, then incubated with primary antibody in blocking buffer for overnight at 4 °C. The primary antibodies used in the assays were anti-β-actin (Cell Signaling Technology, Danvers, MA), anti-fibrinogen (Santa Cruz Biotechnology, Dallas, TX), anti-vimentin (Santa Cruz Biotechnology, Dallas, TX), anti-collagen 1 (Cell Signaling Technology, Danvers, MA) and anti-collagen IV (Southern Biotech., Birmingham, AL). Membranes were then washed four times with PBST and incubated with HRP-conjugated secondary antibody in 5% blocking buffer for 1 h at room temperature. Membranes were again washed four times with PBST. The bands developed by immune-reactive complex were visualized by means of a Chemidoc (Bio-Rad, Hercules, CA). All densitometry analyses of protein bands were performed using ImageJ software (National Institute of Health, Bethesda, MD).

Bioinformatics Analysis. Analytical determination of the investigated proteins (vimentin, Col-IV and fibrinogen) for both physical (direct) and functional (indirect) aspects were analysed through Search Tool for the Retrieval of Interacting Genes/Proteins (STRING, version 11.0) database (https://string-db.org) [11]. Medium confidence (0.400) parameter was chosen for the minimum required interaction score and inflation parameter was set to 3 in MCL clustering. Also, the structure preview inside network bubbles was disabled to simplify the display output. All the proteins were individually searched in STRING database for *Homo sapiens* and *Mus musculus* separately to analyse their interacting partners and their respective functional relevance.

Data Analysis. All values are presented as mean ± standard error mean (SEM). Data analysis was carried out using GraphPad Prism version 6.00 (GraphPad Software, La Jolla, CA) using Student's *t* test or nonparametric Mann-Whitney test, Kruskal-Wallis with Dunn's multiple comparison test and one-way ANOVA with Dunnett's multiple comparison test. However, according to suggestions by Wasserstein et al., the phrase 'statistically significant' has not been used in this study [12].

RESULTS

Systemic Inflammation Induces Modulations in the Expression of ECM Proteins at the Transcript Level in both Local Inflammation Site and Visceral Tissues

Changes in the distribution and modulation of ECM expression have been previously reported in different models of local inflammation, but not much is known about the detailed distribution pattern in systemic inflammation [13]. Therefore, we first investigated the expression dynamics of these ECM proteins at the transcript level using LPS-induced endotoxemia and CLP surgery murine models of systemic inflammation. Following LPS injection and CLP surgery, mesentery, lungs, kidney and liver samples were isolated at 6 h, 12 h, 24 h and 36 h time points, and the mRNA expression was analysed using real-time PCR. Induction of local and systemic inflammation was confirmed by monitoring IL-6 mRNA expression in mesentery and lungs for chosen time points (Supplementary Figure 1). As shown in Fig. 1, Fig. 2 and Supplementary Figure 2, different expression pattern of ECM protein was observed for both LPS and CLP models, possibly due to

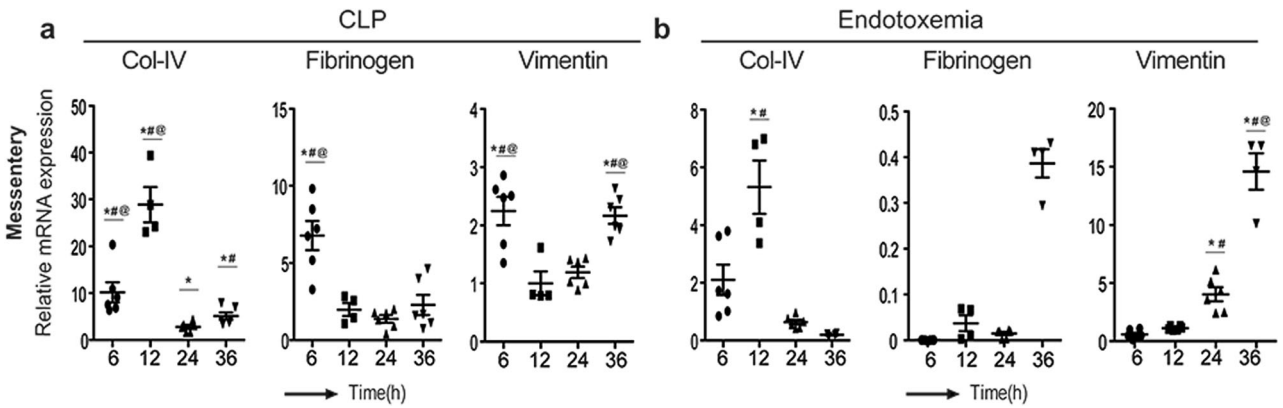

Fig. 1. Expression of ECM proteins at the transcript level in inflammatory site following induction of systemic inflammation. Relative expression kinetics of ECM proteins at mRNA level in mesentery tissues isolated from CLP and endotoxemia animals were measured using quantitative real-time PCR as described in the methods. The dot-plots show the expression of various ECM proteins at mRNA level in mesentery of CLP (**a**) and endotoxemia (**b**) animals. Results are expressed as fold change in comparison to the expression level of respective protein in the mesentery tissues harvested from naïve animals. The data are presented as mean ± SEM ($N = 4$–6/time point) ($p \leq 0.05$, *Student's t test, # one-way ANOVA with Dunnett's multiple comparisons test and @Kruskal-Wallis test with Dunn's multiple comparisons test).

difference in inflammation induction pattern in both models [6, 14]. As shown in Fig. 1a and Supplementary Figure 2a, up to 30-fold increase in the mRNA of Col-IV with a 6- and 4-fold increase in the transcripts of fibrinogen and Col-1, respectively, was observed in the mesentery tissue within 6 h of surgery. A 4-fold increase in the transcripts of laminin-IV was also observed by 12 h post CLP induction.

Upon analysis of tissues from visceral organs, a similar trend for Col-IV was also observed in the lungs (up to 3-fold) and liver (up to 30-fold) with an earlier upregulation of the same in the liver tissues (Fig. 2a, e). In contrast, as shown in Fig. 2c, fibrinogen transcripts showed up to 30-fold increase in CLP surgery group compared to naïve animals. Unlike other three tissues, levels of fibrinogen mRNA were dramatically higher in liver compared to Col-IV. A smaller change of up to 5–6-fold increase in the transcripts of vimentin was also observed in the lungs, liver and kidney tissues of CLP animals (Fig. 2a, c, e).

In LPS model, up to 4- and 10-fold increase in the level of Col-IV was observed in mesentery and liver, respectively, within 6 to 12 h of LPS administration (Figs. 1b and 2f). However, in the lungs and kidney, the upregulation in Col-IV transcript was observed during later time points, *i.e.* 24–36 h following LPS administration (Fig. 2b, d). Elevated levels of fibrinogen transcripts were also observed in the liver and kidney, whereas the upregulation in fibronectin transcript level was limited to lungs only in this model. As shown in Fig. 1b, considerable changes (up to 15-fold) in the expression of vimentin mRNA were also observed within 36 h in the mesentery tissue. So, in both the model of systemic inflammation, Col-IV, fibrinogen and vimentin showed appreciable changes in the expression at transcript levels.

Protein Analysis Shows that Col-IV, Fibrinogen and Vimentin Are Modulated at the Site of Inflammation

To check if the modulations observed in the ECM expression at mRNA levels also reflected at protein level, total protein was extracted from the inflamed mesentery tissues from both CLP- and LPS-induced animals. Expression of various proteins that showed modulation at the transcript levels was analysed using Western blot technique. As shown in Fig. 3a, in the mesentery tissues of CLP animals, up to 2-fold change in the expression of ECM proteins such as Col-IV, fibrinogen and vimentin was observed as early as 6 h post-induction of systemic inflammation and was sustained until 24 h post-surgery. Interestingly, as perceived from the band density, level of fibrinogen protein expression was found to be much more compared to others.

In contrast to CLP model, endotoxemia animals showed a delayed upregulation of both Col-IV and fibrinogen protein in the mesentery tissues that peaked around 24 h following LPS injection (Fig. 4a). However, in line with the CLP model, a 2-fold increase in the expression levels of vimentin was observed within 6 h of LPS injection (Fig. 4a).

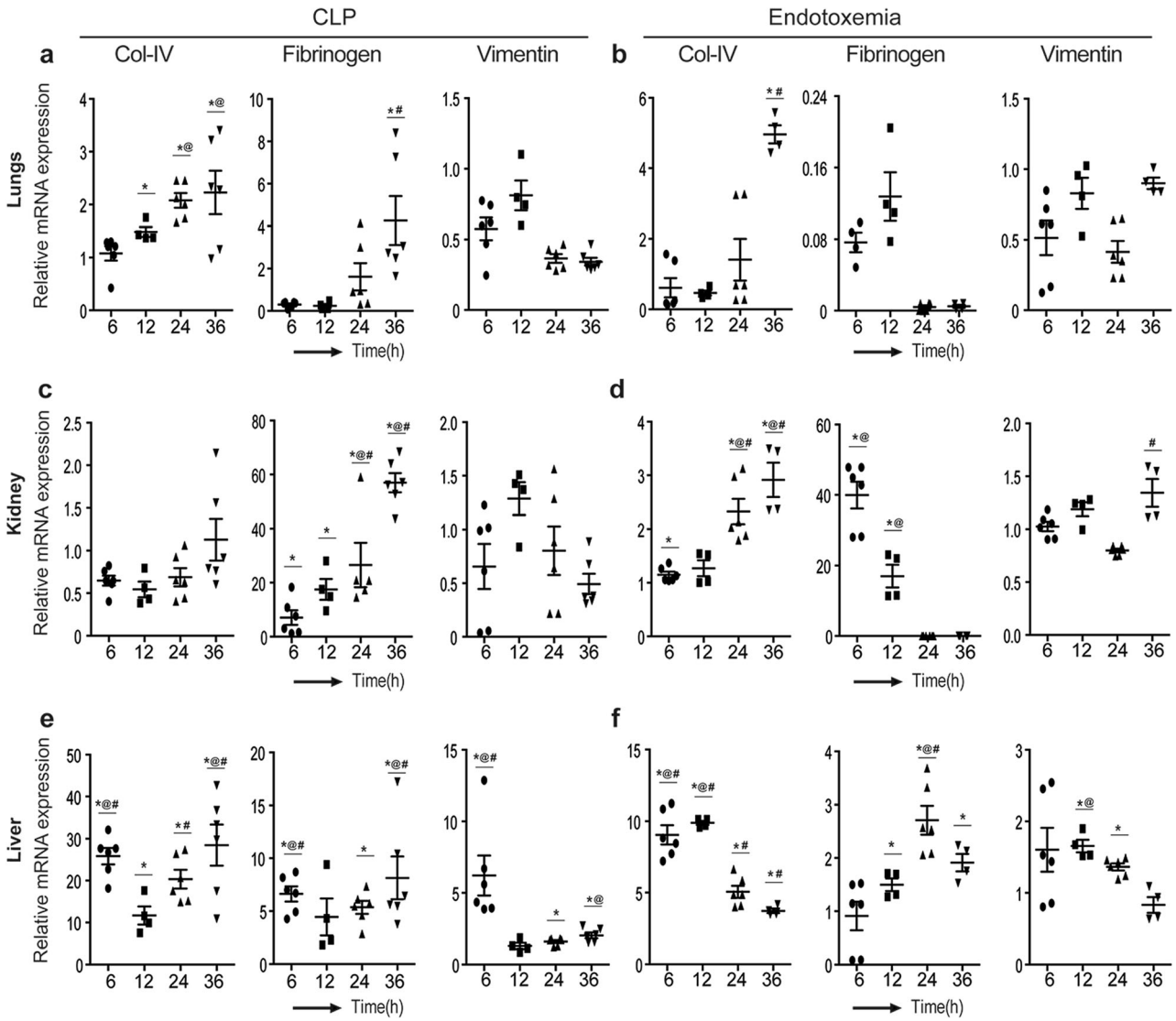

Fig. 2. Expression of ECM proteins at the transcript level in visceral tissues following induction of systemic inflammation. Relative expression kinetics of ECM proteins at mRNA level in lungs, kidney and liver isolated from CLP and endotoxemia animals were measured using quantitative real-time PCR as described in the methods. The dot-plots show the expression of various ECM proteins at mRNA level in visceral organs of CLP (**a**, **c**, **e**) and endotoxemia (**b**, **d**, **f**) animals. Results are expressed as fold change in comparison to the expression level of respective ECM protein in the same tissue harvested from naïve animals. The data are presented as mean ± SEM ($N = 4–6$/time point) ($p \leq 0.05$, *Student's *t* test, #one-way ANOVA with Dunnett's multiple comparisons test and @Kruskal-Wallis test with Dunn's multiple comparisons test).

Modulation of ECM Expression Extends to Visceral Organs During Systemic Inflammation

When a local inflammation is not contained, it spreads throughout the body resulting in systemic inflammation in which visceral organs are also seriously affected. So, to study the modulations in ECM expression in visceral organs, total protein was isolated from lungs, liver and kidney harvested from LPS- and CLP-induced animals, and Western blot was performed. The lungs are one of the vital organs that gets affected relatively quicker following the spread of any infection into the circulation. When analysed at different time points, a moderate upregulation of Col-IV, fibrinogen and vimentin was observed in the lung tissues within

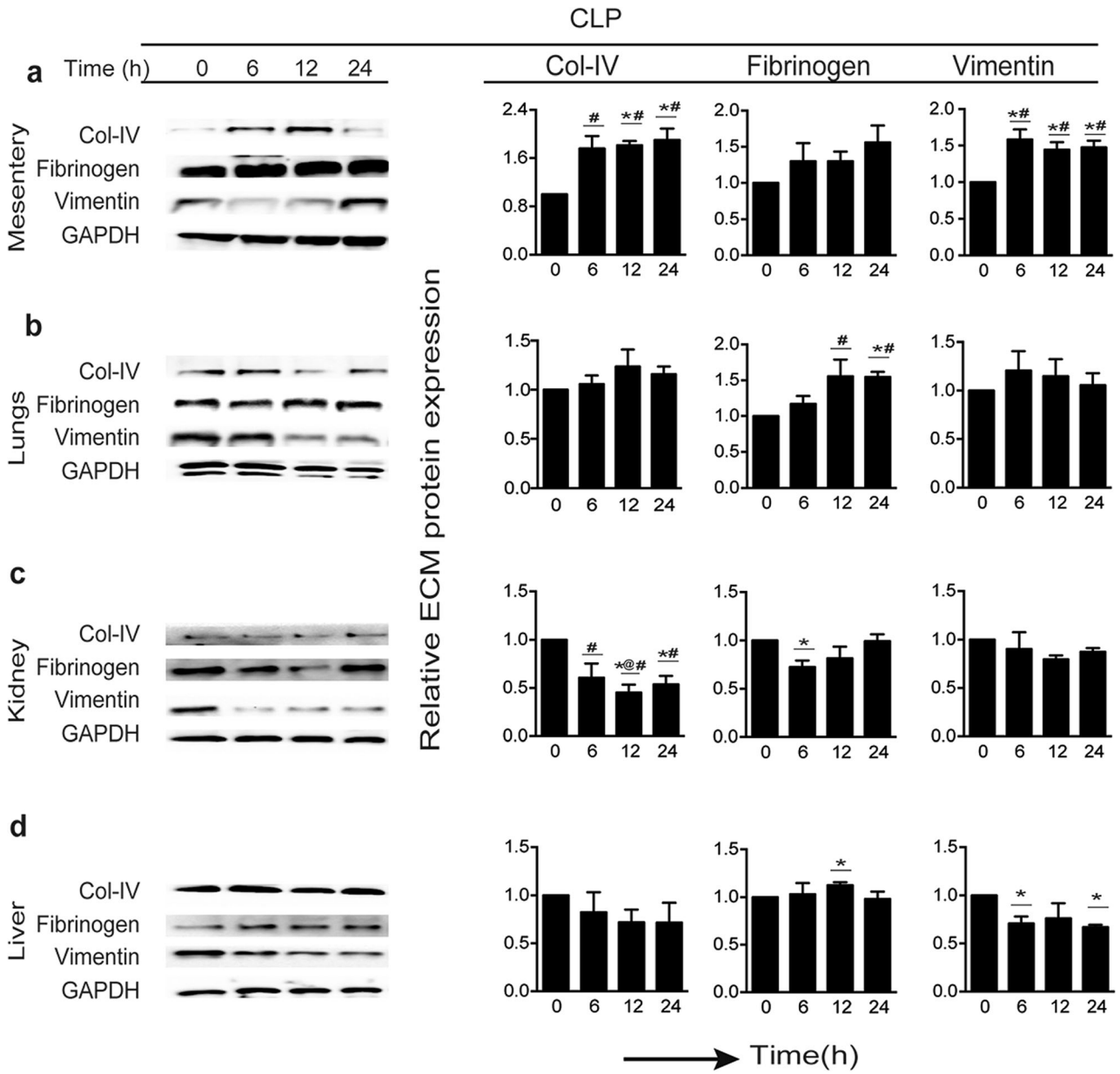

Fig. 3. Expression of ECM proteins at the local inflammation site and distant visceral organs in CLP-induced systemic inflammation. Relative expression of ECM proteins in different tissues at different time points following CLP surgery was measured using Western blot as described in the method section. GAPDH was used as endogenous control for all analysis. The left panels in **a**, **b**, **c** and **d** show the Western blot pictures of respective proteins at different time points in the tissue as labelled in respective figures. The bar diagrams on right panels show the fold change in the expression of specific protein compared to that of corresponding organs of naïve animals as measured by densitometry analysis of individual bands using ImageJ software. The data are presented as mean ± SEM ($p \leq 0.05$, *-Student's *t* test, #one-way ANOVA with Dunnett's multiple comparisons test and @Kruskal-Wallis test with Dunn's multiple comparisons test).

6–12 h post-surgery. Although detected in liver and kidney of inflamed animals, no significant modulations were observed in such tissues in CLP animals.

In contrast to CLP animals, as shown in Fig. 4b, up to 3-fold increase in Col-IV and up to 2-fold upregulation in the expression of fibrinogen and vimentin were observed in

the lung tissues. Possibly, as intense inflammatory response following LPS injection resulted in the modulation of ECM structure of the lung tissues in such animals. Interestingly, as shown in the Fig. 4c, in the kidneys of endotoxemia animals, marked upregulation of fibrinogen was observed as compared to naïve tissues suggesting an important role of this protein in the modulation of inflammatory events. As shown in Fig. 4d, the change in the ECM protein expression in liver was not prominent compared to other tissues.

Bioinformatics Study of Possible Signalling Pathways Associated with Systemic Inflammation

In order to further analyse the possible effects of the modulation in the levels of specified vimentin, Col-IV and fibrinogen, bioinformatics analysis of their respective interacting molecules and the signalling pathways was performed using STRING. The proteins and their interacting molecules were compared both in mouse and human to assess the relevance of the experimental data with respect to inflammatory diseases. The network edges shown in Fig. 5 represent the confidence of the data supported from different sources like text-mining, co-expression and experiments *etc.* where greater thickness of line correlates with better strength of the data support. As depicted in the Fig. 5 (left panel), in human, the vimentin (VIM) showed association with CASP3, CASP7, CASP8, AKT1 protein that were involved in TNF signalling pathway (hsa04668, KEGG pathway) which is very well pronounced in systemic inflammation [15, 16]. Proteins like COL1A1, COL1A2, COL4A2, COL6A1 and ITGA1 were found common in the Col-IV (COL4A1)-mediated ECM receptor (hsa04512, KEGG pathway) and integrin cell surface interactions (HSA-216083, reactome pathway) pathways suggesting its primary association with molecular signals originating from cytoskeletal structures which guide immune cell extravasation and tissue migration. The fibrinogen (FGA) showed interactions with FGB, FGG, F2, F13B and SERPINC1 which mediate the complement and coagulation cascades (hsa04610, KEGG pathway), where notably the communication between FGA and F2 governs the inflammation and possibly is expressed in the tissues for wound-healing purpose [17–19]. As demonstrated in Fig. 5 (left panel), human proteins also showed some unique interactions of CDC5L, AKT1 and STAT3 with vimentin, ITGA1 and COL16A1 with Col-IV, and ALB, HRG with fibrinogen that were absent in mice model.

Similarly, when compared to the murine model, multiple similarities were found in the interacting partners of vimentin, Col-IV and fibrinogen. For example Casp3, Casp7 and Casp8 associated with TNF signalling pathway (mmu04668, KEGG pathway), Col1a1, Col1a2, Col4a2, Col6a1, Itgb1 associated with ECM-receptor (mmu04512, KEGG pathway) and Fgb, Fgg, F2, F13b, Serpinc1, Plg associated with complement and coagulation cascades (mmu04610, KEGG pathway) were found to be some common pathways in both human and mouse. As shown in human protein, murine proteins also showed multiple unique binding partners. In Fig. 5 (right panel), Tmp4, Tnnt3 and Neb specifically interacted with mouse vimentin, Itgb1 and Plod2 with mouse Col-IV and Plg, Apoa2 with mouse fibrinogen proteins.

DISCUSSION

Sepsis-induced multiple organ failure is a prominent pathophysiological condition where excessive and altered migration of inflammatory leukocytes to the tissue is detrimental [20]. In order to migrate through the tissue, leukocytes interact with the ECM *via* their integrin receptors expressed on cell surfaces [21]. The current study shows a parallel comparison between the expression pattern of different ECM proteins such as Col-IV, fibrinogen and vimentin at different time points in different tissues using two different models of systemic inflammation having separate mechanisms and distinct pattern for onset of inflammatory events [6, 14, 22]. Our data shows more prominent modulations at the site of inflammation and the lung tissues compared to liver and kidney with different thresholds in both the models.

Previous studies have shown that inflammation-induced ECM remodelling could modulate the severity of inflammatory diseases by altering immune cell migration and functions [3, 23]. Studies also indicate that tissue-specific expression of ECM proteins could modulate the abnormal influx of inflammatory cells into the local site as well as distant visceral tissues. Data presented in the current study shows that both CLP and endotoxemia models demonstrated a unique time- and tissue-specific changes in the expression of ECM proteins. Interestingly, in endotoxemia, although the modulation in ECM protein expression was less intense at the inflammation site, it has more intense changes in the lungs when compared to the same proteins in CLP suggesting an inflammatory stimulation-dependent modulation of inflammatory cells. This contrasting observation between CLP and

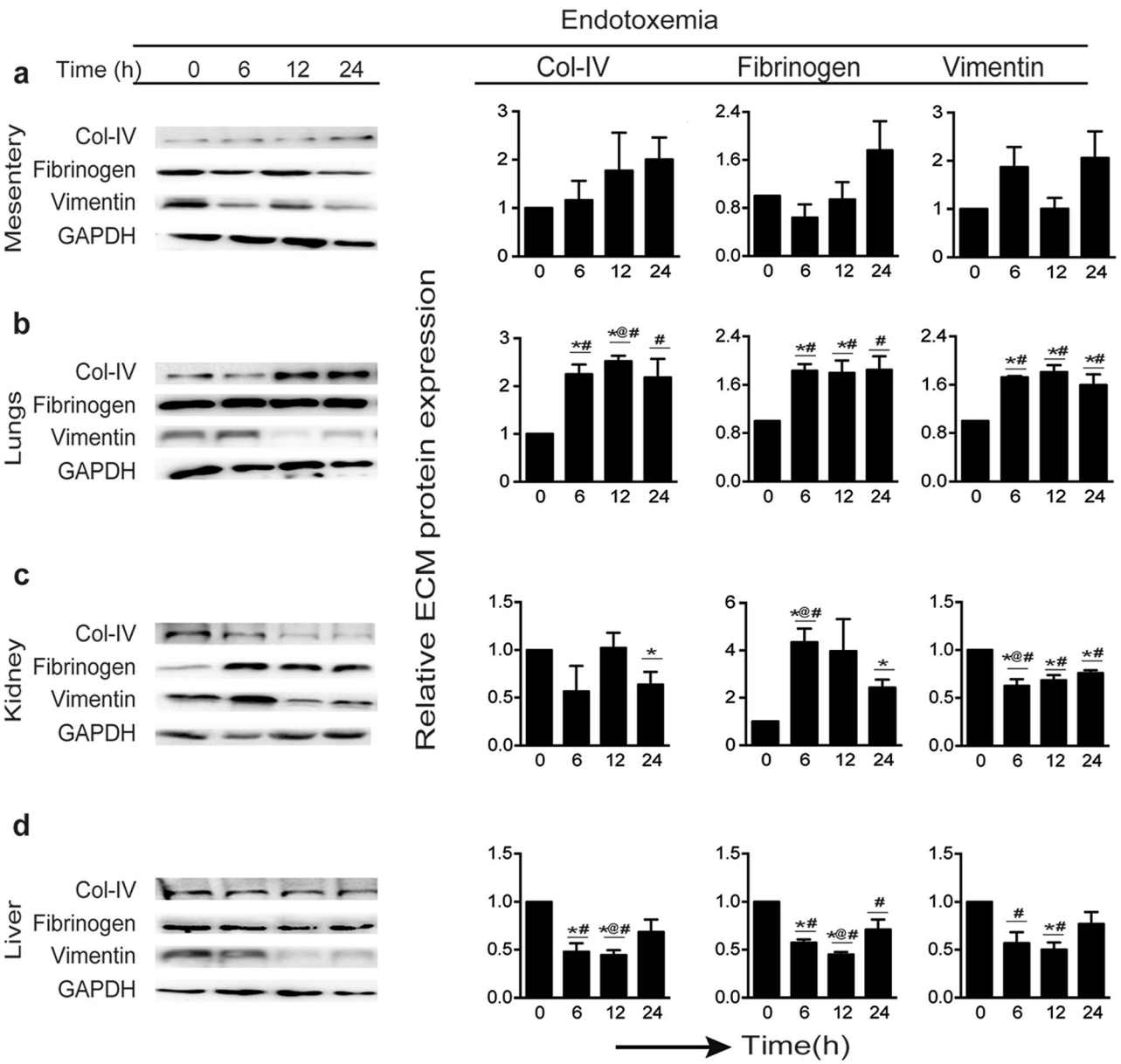

Fig. 4. Expression of ECM proteins at the local inflammation site and distant visceral organs in LPS-induced endotoxemia. Relative expression of ECM proteins in different tissues at different time points following LPS injection was measured using Western blot as described in the method section. GAPDH was used as endogenous control for all analysis. The left panels in **a**, **b**, **c** and **d** show the Western blot pictures of respective proteins at different time points in the tissue as labelled in respective figures. The bar diagrams on right panels show the fold change in the expression of specific protein compared to that of corresponding organs of naïve animals as measured by densitometry analysis of individual bands using ImageJ software. The data are presented as mean ± SEM ($p \leq 0.05$, *Student's *t* test, #one-way ANOVA with Dunnett's multiple comparisons test and @Kruskal-Wallis test with Dunn's multiple comparisons test).

endotoxemia may be the result of rapid dissemination of LPS throughout the body causing a more complex immune response compared to CLP, where the diffusion of polymicrobial infection is comparatively slow. Previous studies have also demonstrated that, instead of having comparable mortality levels, it could be possible that the inflammatory components such as cellular infiltrates, cytokines, chemokines, proteases, reactive oxygen and

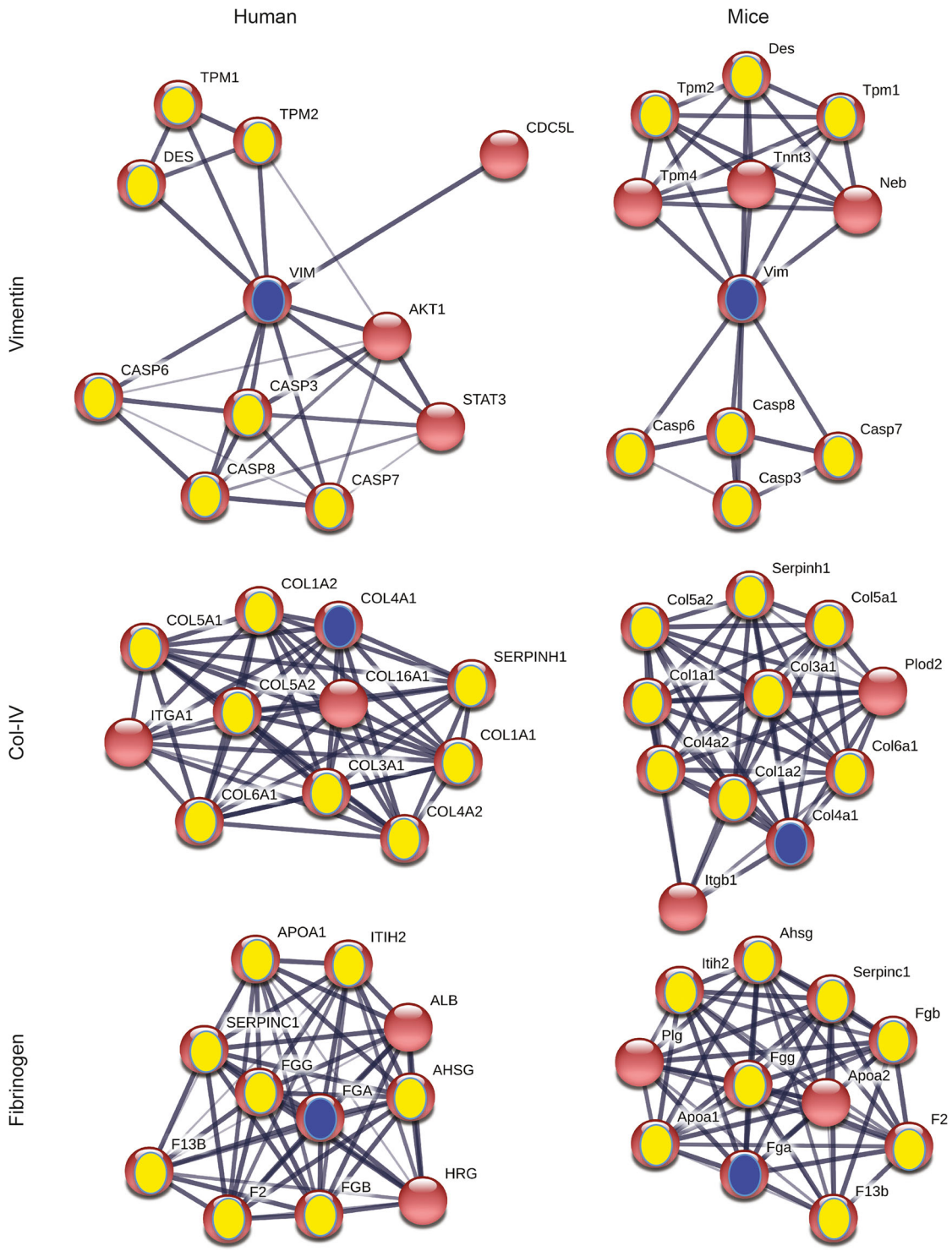

Fig. 5. Protein-protein interaction (PPI) network of investigated ECM proteins. Vimentin, Col-IV and fibrinogen interacting PPI network for human (left panel) and mice (right panel) acquired from STRING v11.0. The network edges show the confidence where darker line thickness reflects better strength of the data support.

nitrogen species could be differentially expressed at various time points in CLP and endotoxemic animals, which could contribute to the different expression pattern of ECM proteins in both the models [6, 14, 22]. Alternately, these changes in matrix proteins may also influence the differences seen in the inflammatory cell influx and secreted mediators from such cells in the peritoneum and other visceral tissues in both the models [6].

Fibrinogen, a 340-kDa multimeric glycoprotein, has an important role in vascular homeostasis. Although fibrinogen is normally present at a basal level in blood, during inflammation, its concentration increases and serves as an important biomarker for inflammation [24]. Previous study in acute phase response showed the role of fibrinogen as an adhesive protein for the intra-alveolar aggregation and adherence of *P. carinii* and subsequent immune response causing severe tissue damage [25]. Fibrinogen is also responsible for chemotaxis of pro-inflammatory neutrophils and macrophages *via* binding with integrin $\alpha_M\beta_2$ in inflammatory conditions [17]. It also participates in repair mechanism where it binds to macrophage causing secretion of MMP-12 that mediates wound healing [18]. Our study showed upregulation of fibrinogen at both mRNA and protein level at the site of inflammation as well as visceral organs. As a matter of fact, fibrinogen was one of the highly expressed proteins as compared to others when assessed from the density of the bands with equal protein loading. Further bioinformatics analysis on interaction of fibrinogen with other molecules revealed the possibility of FGA-F2 interaction which is reported to be involved in the healing process. Possibly, the upregulation in fibrinogen promotes chemotaxis of inflammatory immune cells, thus exaggerated immune response, tissue damage and multi organ failure. On the other hand, the possibility of role of fibrinogen in the healing mechanism cannot be ruled out.

Vimentin is a cytoskeletal protein that helps in maintaining the cell structure and integrity. It is also associated with regulation of cell migration and invasion [26]. Additionally, it plays a crucial role in inflammation, where it is responsible for NLRP-3 activation and transcellular migration of immune cells to damage site [27]. The direct interaction of vimentin with integrin β_1 is also known to facilitate the binding of $\alpha_5\beta_1$ to fibronectin [28]. Our data shows upregulated expression of vimentin in the lungs and mesentery during sepsis. Bioinformatics analysis showed the interaction of vimentin with different signalling molecules such as CASP3, CASP7, CASP8 and AKT1 which are involved in the TNF signalling pathway responsible for regulation of cell survival. In accordance with previous reports, our data suggests the possibility of role of vimentin in increasing the severity of the disease by mediating the infiltration of inflammatory immune cells to organs.

Col-IV is a vital component of the basement membrane responsible for its integrity and stability. During inflammation, destruction of basement membrane by MMPs secreted by infiltrated immune cells is responsible for generation of matrikines that have chemotactic effect for variety of immune cells [29, 30]. Moreover, it is also involved in transmigration of neutrophils through basement membrane by binding with L-selectin expressed on immune cells [31]. Further bioinformatics analysis by STRING revealed the possible interaction of Col-IV and integrin suggesting its possible association with transmigration and interstitial migration inside tissue. We observed high Col-IV expression mainly in lungs and mesentery of septic animals. This upregulation might be involved in the infiltration of inflammatory neutrophils, which causes further damage to the affected organs.

Previously, the changes in Col-IV, fibrinogen, vimentin, laminin *etc.* were reported in brain injury, lung injury, arthritis, cancer *etc.* [2, 8]. These investigations on altered ECM distribution and expression were only limited to local inflammatory condition. The current report using two well-characterized mouse models shows the dynamic changes of ECM expression during systemic inflammation. The dynamic changes might be responsible for infiltration/chemotaxis and downstream function of immune cells that can play crucial role in driving the disease severity. The inflammatory events in human might differ from mice up to some extent. However, our bioinformatics analysis showed interactions of the reported proteins with molecules involved in signalling pathways associated with systemic inflammation, which were common to human and mice. Moreover, extensive study on the functional relevance of the bioinformatics data is needed for validation. Further research and development based on the current findings will help more to understand the complex pathophysiology of sepsis and may lead to development of a new therapeutic approach.

AUTHOR'S CONTRIBUTION

PC, SD, CB and PD performed the *in vivo* experiments. CB and SD performed qRT-PCR and Western blot experiments and analysed the data. PD and PC assisted in RNA isolation from animal tissues and performing experiments. PK performed the bioinformatics studies and analysed the data. PPS conceived and directed the study. CB, SD and PPS wrote the manuscript.

FUNDING INFORMATION

The Department of Biotechnology, Govt. of India (102/IFD/SAN/1671/2014-2015) to P.P.S., University Grants Commission, Govt. of India fellowship to CB (Sr. No-2061330682) and SPD (Sr. No-2061430670), Indian Council of Medical Research, Govt. of India fellowship to PD, and, Ministry of Human Resource Development, Govt. of India fellowship to PC (MHR 02-23-200-429) provided funding.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest. The authors declare that they have no conflicts of interest.

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