American Journal of Pathology, Vol. 159, No. 4, October 2001

Short Communication

Molecular Signatures of Sepsis

Multiorgan Gene Expression Profiles of Systemic Inflammation

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During sepsis the host's system-wide response to microbial invasion seems dysregulated. Here we explore the diverse multiorgan transcriptional programs activated during systemic inflammation in a cecal ligation/puncture model of sepsis in rats. Using DNA microarrays representing 7398 genes, we examined the temporal sequence of sepsis-induced gene expression patterns in major organ systems including lung, liver, kidney, thymus, spleen, and brain. Although genes known to be associated with systemic inflammation were identified by our global transcript analysis, many genes and expressed sequence tags not previously linked to the septic response were also elucidated. Taken together, our results suggest activation of a highly complex transcriptional response in individual organs of the septic animal. Several overlying themes emerged from our genome-scale analysis that includes 1) the sepsis response elicited gene expression profiles that were either organ-specific, common to more than one organ, or distinctly opposite in some organs; 2) the brain is protected from sepsis-induced gene activation relative to other organs; 3) the thymus and spleen have an interesting cohort of genes with opposing gene expression patterns; 4) genes with proinflammatory effects were often balanced by genes with anti-inflammatory effects (eg, interleukin-1β/decoy receptor, xanthine oxidase/superoxide dismutase, Ca^{2+}-dependent PLA_2/Ca^{2+}-independent PLA_2); and 5) differential gene expression was observed in proteins responsible for preventing tissue injury and promoting homeostasis including anti-proteases (TIMP-1, Cpi-26), oxidant neutralizing enzymes (metallothionein), cytokine decoy receptors (interleukin-1RII), and tissue/vascular permeability factors (aquaporin 5, vascular endothelial growth factor). This global perspective of the sepsis response should provide a molecular framework for future research into the pathophysiology of systemic inflammation. Understanding, on a genome scale, how an organism responds to infection, may facilitate the development of enhanced detection and treatment modalities for sepsis.
(Am J Pathol 2001, 159:1199–1209)

The systemic inflammatory response seems to be initiated by the release of bacterial lipopolysaccharide or other microbial substances into the lymphatics and circulation. Once the sepsis cascade is triggered, an unregulated systemic response ensues that can progress to multiple organ failure. Sepsis-induced multiple organ failure is associated with a high mortality rate in humans and is characterized clinically by profound pulmonary, cardiovascular, renal, and gastrointestinal dysfunction.[1,2] The systemic appearance of diverse biological mediators plays a central role in the pathophysiology of sepsis including microbial signal molecules, cytokines, complement activation products, coagulation factors, and cell-adhesion molecules.[3,4] Although a sepsis-like state can be induced experimentally by infusion of lipopolysaccharide or live bacteria, cecal ligation puncture (CLP) in rodents mimics many features of the septic state in humans.[5] Animals develop progressive bacteremia, appearance of multiple cytokines and chemokines in plasma, fever, hypermetabolism, and other clinical features analogous to those found in humans with sepsis.[6]

DNA microarray (or DNA chip) technology is promising to revolutionize the way fundamental biological questions are addressed in the postgenomic era. Rather than the traditional approach of focusing on one gene at a time,

Supported by the National Institutes of Health (grants HL-31963 and GM-29507 to P. A. W.).

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Accepted for publication June 18, 2001.

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genomic-scale methodologies allow for a global perspective to be achieved. DNA microarrays have successfully been used to molecularly classify cancers, identify single nucleotide polymorphisms, genotype viruses, and monitor patterns of coordinated gene expression after a variety of biological stimuli. Obtaining large-scale gene expression profiles of cancers should theoretically allow for the identification of subsets of genes that function as prognostic disease markers or biological predictors of a therapeutic response. Oligonucleotide chips have been used for the molecular classification of acute leukemias,[7] demonstrating the feasibility of using microarrays for identifying new cancer classes and for assigning tumors to known classes. Similarly, diffuse large B-cell lymphoma has been dissected into two prognostic categories by gene expression profiling.[8]

Although numerous studies have been undertaken to assess global gene expression patterns in cancer,[7–10] few have been used in the context of inflammation or sepsis. In a proof of concept study, a 1000-element DNA microarray has been used to analyze gene expression changes in cytokine-activated monocytes, synovial fluid specimens from patients with rheumatoid arthritis, and intestinal mucosa biopsies from patients with inflammatory bowel disease.[11] A similar global expression profiling study was performed characterizing transcript alterations in the lung using a rodent model of pulmonary fibrosis.[12]

As described above, a major complication of septic patients is development of acute respiratory distress syndrome and onset of multiple organ failure. It has been demonstrated both experimentally and clinically that sepsis causes the appearance in plasma of a series of cytokines, such as interleukin (IL)-1, tumor necrosis factor (TNF)-α, and IL-6. This phenomenon seems to place organs (liver, lung, and kidney) at risk of injury and failure. Why these organs become targets of injury during sepsis is poorly understood. Characterizing the molecular fingerprint (or gene profile) of sepsis in this context may help elucidate the mechanism of sepsis-induced multiple organ failure and suggest further approaches for therapeutic intervention.

In the present study, we developed an 8064 element (8K) rat cDNA microarray to analyze multiorgan/multisystem gene expression patterns in a well-characterized rat CLP model of sepsis.[13,14] We propose that the response to sepsis induces both distinct and shared gene expression programs in various organs—perhaps to minimize tissue injury by the host's own immune system. Usually, these mediators are measured in plasma in the face of a very dynamic and rapidly changing environment of sepsis. Extrapolations to individual organs is not possible. Our hypothesis is that microarray analysis of genes expressed in organs during sepsis may be predictive of outcome, especially in organs that are known to be compromised during sepsis. Such studies may provide important insight into multiorgan failure during sepsis. Although several studies have successfully used DNA microarrays to molecularly classify malignancies,[7,8] this is the first gene-profiling study to address an important disease process at a multiorgan, multisystem level.

Materials and Methods

Rat Model of Sepsis

Sepsis was induced in rats by CLP as described in detail elsewhere.[13,14] Briefly, male Long-Evans-specific pathogen-free rats (275 to 300 g; Harlan, Indianapolis, IN) were used in all studies. Anesthesia was induced by intraperitoneal administration of ketamine (20 mg/100 g body weight). Through a 2-cm abdominal midline incision, the cecum was ligated below the ileocecal valve without obstructing the ileum or colon. The cecum was then subjected to a single through and through perforation with a 21-gauge needle. After repositioning the bowel, the abdominal incision was closed with plain surgical suture 4-0 and metallic skin clips. Sham-operated rats underwent the same procedure except for ligation and puncture of the cecum. Lung, liver, thymus, spleen, kidneys, and brain were harvested from CLP rats, sham-rats, and control untreated rats. Various time points (6, 12, 18, and 24 hours) after surgery were used in the CLP and sham animals. Organs from three rats from each condition were pooled, snap-frozen, and stored at −80°C.

Microarray Analysis

DNA microarray analysis of gene expression was done essentially as described by the Brown and Derisi Labs (available at www.microarrays.org). The sequence-verified cDNA clones on the rat cDNA microarray are listed in the Supplementary Information and are available from Research Genetics (www.resgen.com). Purified polymerase chain reaction products, generated using the clone inserts as template, were spotted onto poly-L-lysine-coated microscope slides using an Omnigrid robotic arrayer (GeneMachines, CA) equipped with quill-type pins (Majer Scientific, AZ). One full print run generated ~100 DNA microarrays. All chips have various control elements, which include human, rat, and yeast genomic DNAs, standard saline citrate, yeast genes, housekeeping genes, among others. In addition, we have separately obtained ~500 inflammation- and apoptosis- related cDNAs from Research Genetics to serve as independent controls for clone tracking and function as duplicates for quality control. Protocols for printing and postprocessing of arrays are available in the public domain (www.microarrays.org) and described previously.[15]

Pooled rat organs were homogenized and poly-(A)+ mRNA was isolated using a commercial kit (Fasttrack 2.0; Invitrogen, Carlsbad, CA). Once isolated, mRNA was used as a template for cDNA generation using reverse transcriptase. Inclusion of amino allyl-dUTP in the reverse transcriptase reaction allowed for subsequent fluorescent labeling of cDNA using monofunctional N-hydroxy succinimidyl (NHS) ester dyes (as described at www.microarrays.org). In each experiment, fluorescent cDNA probes were prepared from an experimental mRNA sample (Cy5 labeled) and a control mRNA sample (Cy3 labeled) isolated from untreated, control rat organs. For example, lung isolated from CLP and sham rats was compared against control lung from untreated rats (other

organs were compared similarly). The experimental cDNA sample was coupled to a monofunctional Cy5-NHS ester and the reference cDNA sample to a Cy3-NHS ester (Amersham, Arlington Heights, IL). The labeled probes were then hybridized to 8K rat cDNA microarrays. Fluorescent images of hybridized microarrays were obtained using a GenePix 4000A microarray scanner (www.axon.com; Axon Instruments, CA).

Data Analysis

Primary analysis was done using the Genepix software package. Images of scanned microarrays were gridded and linked to a gene print list. Initially, data are viewed as a scatter plot of Cy3 *versus* Cy5 intensities. Cy3 to Cy5 ratios are determined for the individual genes along with various other quality control parameters (eg, intensity over local background). The Genepix software analysis package flags spots as absent based on spot characteristics (refer to web site). Additionally, bad spots or areas of the array with obvious defects were manually flagged. Spots with small diameters (<50 μm) and spots with low signal strengths <350 fluorescence intensity units over local background in the more intense channel were discarded. Flagged spots were not included in subsequent analyses. Data were scaled such that the average median ratio values for all spots were normalized to 1.0 (done separately for each array). An arbitrary cut-off ratio of twofold was used to select genes as significantly up- or down-regulated relative to the control sample.

Normalized fluorescence ratios of nonflagged array elements were uploaded to a Microsoft Access Database (Microsoft, WA). The data sets for each organ were individually queried for genes that were differentially expressed in the CLP organs as compared to control organs (ratios >2.0 or <0.5) but not in the sham-operated organs (ratios between 0.5 and 2.0). The data sets from individual organ analyses were then combined and imported into M. Eisen's Gene Cluster Program and array elements that were not represented in at least 75% of the experimentals were excluded. The data were log2 transformed and hierarchically clustered with average linkage clustering and visualized using the TreeView Program.[16] In some cases, inclusion thresholds were increased to focus the returned clusters.

Northern Blot Analysis

Five μg of poly A+ RNA were resolved by denaturing formaldehyde-agarose gel and transferred onto Hybond membrane (Amersham) by a capillary transfer set up. Hybridizations were performed by the method described by Church and Gilbert.[17] Briefly, prehybridization was performed for 1 hour at 65°C in a solution containing 1% bovine serum albumin (fraction V), 8% sodium dodecyl sulfate, 0.5 mol/L phosphate buffer, pH 7.0, and 1 mmol/L ethylenediaminetetraacetic acid, pH 8.0. Hybridization was performed in prehybridization buffer for 16 hours at 65°C after adding the denatured probe at 2 to 3 $\times$ 10^6 cpm/ml concentration. Blots were washed with 2$\times$ standard saline citrate/0.1% sodium dodecyl sulfate at room temperature three times for a period of 30 minutes. Subsequently the blots were washed twice in 0.2$\times$ standard saline citrate/0.1% sodium dodecyl sulfate at room temperature at 65° twice for 10 minutes each. Signal was visualized and quantitated by phosphorimager. For relative fold estimation, the ratio of the intensity of the respective transcript in the CLP animal over the transcript intensity in the sham animal was determined. Similarly, for microarray analysis, the normalized Cy5/Cy3 ratio of the transcript in CLP animals is compared to the Cy5/Cy3 ratio in sham animals.

Results

Profiling Sepsis Using High-Density Rat cDNA Microarrays

The glass slide cDNA microarrays developed here include ~2000 known, named genes from the Research Genetics rat cDNA clone set, 5000 expressed sequence tags (ESTs), and 500 control elements (which include genomic human, rat, and yeast DNAs, yeast genes, and so forth). We also included a separate set of ~500 inflammation-related genes to serve as replicates on the microarray and provide internal controls for reproducibility of gene expression quantitation (See Supplementary Information for the complete annotated list of these cDNAs). Using this 8K-rat microarray, we profiled gene expression across multiple time points in the lung, liver, kidney, spleen, thymus, and brain of CLP and sham-operated rats. Organs were pooled from at least three rats for each time point of the study, thus minimizing variation between animals. Fluorescently labeled (Cy5) cDNA was prepared from mRNA from each experimental sample. For each organ, a reference sample was prepared from three unoperated, untreated rats (control) and labeled using a second distinguishable fluorescent nucleotide (Cy3).

In all, more than 40 8K rat cDNA microarrays were used to assess gene expression in six different tissues (120 rat organs) at four time points (6, 12, 18, and 24 hours) during CLP-induced sepsis. Figure 1 provides an overview of the variation in gene expression across different organs/systems. Scatter plots of Cy5 *versus* Cy3 intensities are shown for each organ at an early time point (6 hours) and at a late time point (24 hours) of CLP-induced sepsis. As expected, control lung cDNA labeled with Cy5 compared against control lung cDNA labeled with Cy3 revealed a strong linear relationship ($R^2 = 0.97$). Organs harvested from septic animals, however, displayed various increases in scatter with R^2 varying from 0.89 (24-hour septic brain) to 0.43 (24-hour septic liver). Differential gene expression was greatest in the early and late time points of the sepsis liver ($R^2 = 0.50$ at 6 hours, $R^2 = 0.43$ at 24 hours), an organ known to produce large quantities of acute phase reactants. Interestingly, the brain had very limited changes in gene expression during sepsis ($R^2 = 0.86$ and 0.89), presumably because of the blood-brain barrier that prevents passage of blood components into brain tissue.

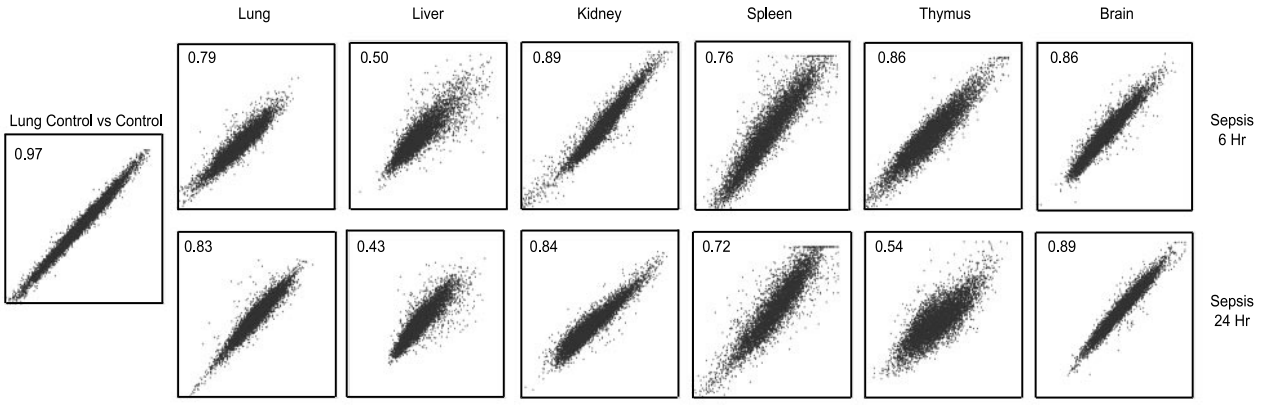

Figure 1. Scatter plot representations of gene expression changes in multiple organs during sepsis. Green (Cy3) intensity ranging from 100 to 10^5 is shown in log scale on the **horizontal axes**, whereas (Cy5) intensity is similarly represented on the **vertical axes**. Respective reference samples consisted of control organs (from untreated rats) and were labeled with Cy3, whereas the experimentals consisted of organs harvested from CLP rats at the 6- and 24-hour time points and were labeled with Cy5. Control lung (labeled with Cy5) compared to itself (labeled with Cy3) displayed a strong linear relationship ($R^2 = 0.97$). By contrast, when organs from septic animals were compared to respective control organs, differential gene expression was observed (with R^2 varying form 0.43 to 0.89). Organs from three animals were combined before mRNA extraction for each time point to minimize animal to animal variation. Microarray hybridizations were done using an 8K rat cDNA chip. Linear fit R^2 value for each scatter plot is provided.

Clustering of Gene Expression Patterns Induced by Sepsis

Organs harvested from sham-operated rats displayed numerous changes in gene expression when compared to organs from control rats (see Supplementary Material). Sham animals underwent the same procedure as CLP animals except for ligation and puncture of the cecum and thus, these gene expression patterns are likely characteristic of the sham operation that includes anesthesia, abdominal incision, and closure. Although anesthesia- and surgery-induced changes are of interest, the primary focus of this study was to monitor changes induced by the sepsis state. Thus, the gene expression data sets (sham and experimentals) for each organ were queried for genes that had at least a twofold or higher variation of the Cy5/Cy3 ratio in the septic sample but not in the matched sham samples. As sepsis induces a systemic response involving multiple organs, we chose to explore the gene expression data using a hierarchical clustering format in which intraorgan and interorgan relationships could be evaluated (Figure 2). The colored bars on the right of Figure 2 indicate clusters of coordinately expressed genes highlighting interrelationships between organ systems. For example, cluster A includes genes that were up-regulated in most if not all of the tissues profiled. By contrast, cluster H highlights genes that were coordinately down-regulated in both the lung and the spleen. Interestingly, there were even groups with discordant gene expression, as exemplified by cluster E, which includes genes that were up-regulated in the spleen but down-regulated in the thymus. Organ-specific gene expression alterations are also evident in clusters B, F, and L (Figure 2). Taken together, Figure 2 illustrates the diverse and interrelated gene expression patterns of a whole organism responding to a systemic inflammatory stimulus (CLP). The entire data set underlying Figure 2 can be obtained from the Supplementary Materials.

Several of the sepsis clusters shown in Figure 2, were examined in more detail (Figure 3). Cluster A is especially interesting because it harbors genes that were up-regulated in most of the organs profiled, defining a set of genes with a relatively universal response to sepsis. As expected, well-known mediators of the inflammatory response are included in cluster A including IL-1β, phospholipase A_2 (PLA_2), and complement components C1q and C3. IL-1β has a central role in the pathogenesis of sepsis and mediates its proinflammatory effects by binding to its cognate receptor (IL-1R) and subsequently activating the transcription factor nuclear factor-κB.[18] By microarray analysis, IL-1β transcript was found to be increased in all of the organs tested excluding the brain. Interestingly, IL-1 receptor type II (IL-1RII), which functions as a decoy receptor for IL-1,[19,20] was shown to be up-regulated in a similar set of tissues (Figure 3). Cluster A also includes PLA_2, an important mediator of arachidonic acid metabolism and recently implicated in the pathogenesis of sepsis-induced lung injury.[21] Because its transcript was increased in most of the tissues we examined, PLA_2 may have a more encompassing role in sepsis-induced tissue injury than previously recognized. Many other known inflammatory markers are contained in cluster A, including complement proteins, proteases, anti-proteases, and oxidant scavenging enzymes, among others. These mediators are discussed in more detail in the context of the functional clusters described in Figure 4. In addition to uncharacterized ESTs, there are numerous genes that were identified by our screen but not previously known to be involved in the sepsis response. For example, a member of cluster A, n-chimaerin, a p21rac-GTPase-activating protein and phorbol ester receptor[22] presumed to have an important role in neuronal signaling, is highly induced in many of the tissues from animals with sepsis. N-chimaerin has previously been shown to enhance the biological activities of p21rac, including actin cytoskeletal mobilization and superoxide generation (in conjunction with NADPH oxidase assembly).[23] However, the precise role of n-chimaerin up-regulation during sepsis is unclear. A number of previously uncharacterized ESTs are also presented in this cluster.

Guilt by association would suggest that they potentially have a role in the septic response.

Cluster H (Figure 3) depicts genes that are repressed during the response to sepsis, an area that has received little attention in the study of inflammation. Specifically, these are transcripts that are coordinately decreased in the lung and spleen. Several genes in this cluster are involved in maintaining the extracellular matrix and are repressed during sepsis, including pro-α2 collagen I, pro-α1 collagen Type III, tenascin X, and protein-lysine 6-oxidase. Genes involved in maintaining the extracellular matrix presumably have more of a role in tissue repair and/or chronic inflammation than in sepsis, at least during the time interval studied. Aquaporin 5, a gene involved in alveolar fluid clearance,[24] was consistently down-regulated in the CLP lung and thus may account for extravascular fluid accumulation in the injured lung.[25] It is also interesting to note that Ca^{2+}-independent PLA_2 and phospholipase D are markedly repressed in a number of tissues, which is in stark contrast to increased cytoplasmic PLA_2 transcript levels (Figure 3, cluster A).

The spleen and thymus express an interesting set of genes (cluster E) that collectively exhibited diametrically opposed patterns of gene expression. Tyrosine/tryptophan monooxygenase (14-3-3 eta) is one member of this cluster and is involved in cell signaling mediated by Ca^{2+}/calmodulin-dependent protein kinases and protein kinase C.[26] This member of the 14-3-3 family may also function as an inhibitor of apoptosis by repressing the activity of p38MAP kinase.[27] Interestingly, our previous work in CLP rats demonstrated marked decreases in thymic weight correlating with thymocyte apoptosis that was not the case in the spleen or the other organs tested.[28] An interesting pair of genes in this cluster are ESTs highly similar to HSP-90 β and the p59 protein (FK506-BP4), which have been shown to physically interact, and in concert, modulate intracellular trafficking of steroid hormone receptors.[29,30] Both proteins exhibit increased transcript expression in the spleen and decreased expression in the thymus. Platelet-activating factor is a bioactive phospholipid with numerous proinflammatory activities including increasing vascular permeability and promoting leukocyte aggregation, adhesion, and chemotaxis. Of note, cluster E contains an EST with high similarity to the platelet-activating factor acetylhydrolase, an enzyme that functions to inactivate platelet-activating factor. The reason for discordant expression of this transcript in the thymus and the spleen remains to be determined.

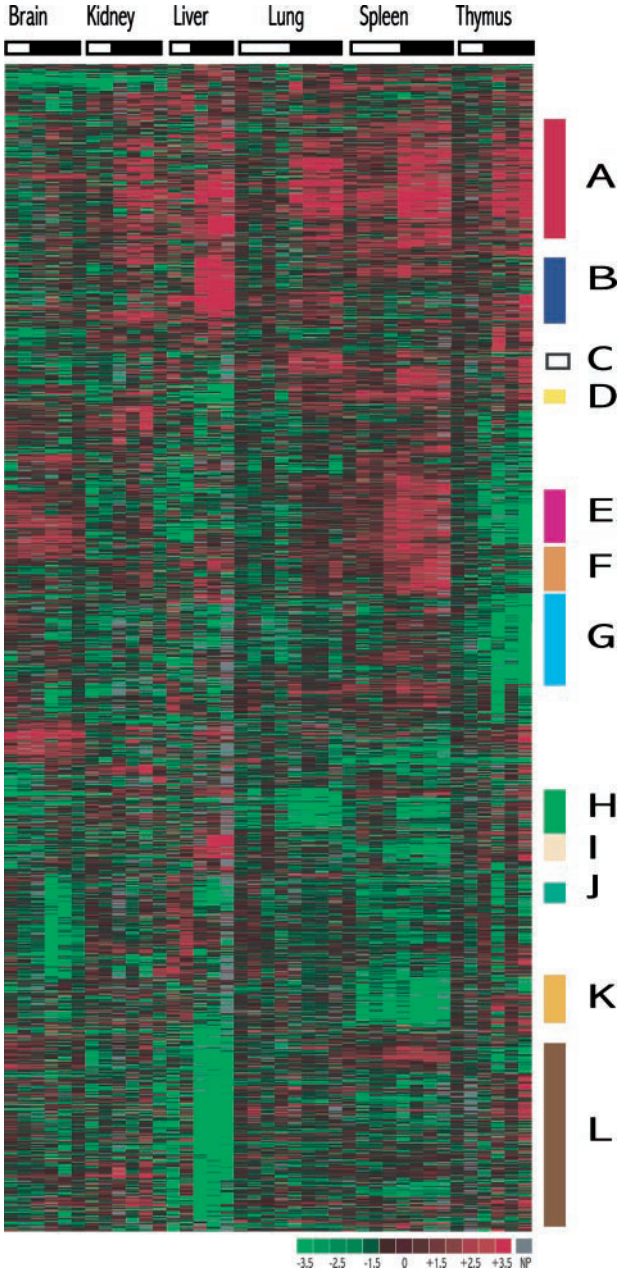

Figure 2. Peritonitis and sepsis induced by CLP initiated diverse gene expression patterns in a rat sepsis model that were captured by microarray analysis and cluster representation. Gene expression was monitored in six organs at various times points (6, 12, 18, and 24 hours) in the CLP rat model. Hierarchical clustering of the data identified distinct patterns of gene expression in the organs studied. Each column represents a different experimental organ sample with sham-operated samples under the **white bars** and CLP/sepsis samples under the **black bars**. Time points (ranging from 6 hours to 24 hours) are ordered from left to right. Each row represents a single gene with ~1500 genes depicted. The results represent the ratio of hybridization of fluorescent cDNA probes prepared from each experimental mRNA to an untreated control organ mRNA sample. These ratios are a measure of relative gene expression in each experimental sample and are depicted according to the color scale at the bottom. Red and green colors in the matrix represent genes that are up- and down-regulated, respectively, relative to the untreated control organ. **Black lines** in the matrix represent transcript levels that are unchanged, whereas **gray lines** signify technically inadequate or missing data (NP, not present). Color saturation reflects the magnitude of the ratio relative to the median for each set of samples. Approximately 80% of the scaled-down cluster is shown here. **Colored bars** on the **right**, highlight clusters of special interest as follows: **cluster A**, gene expression that is up-regulated in most of the tissues studied; **cluster B**, genes with increased expression in the liver; **cluster C**, genes with increased expression in the lung and thymus; **cluster D**, genes with decreased expression in the liver and increased expression in the spleen; **cluster E**, genes with increased expression in the spleen and decreased expression in the thymus; **cluster F**, genes increased primarily in the spleen; **cluster G**, genes with decreased expression in the spleen; **cluster H**, genes with decreased expression in the lung and spleen; **cluster I**, genes with increased expression in the liver and decreased expression in the spleen; **cluster J**, genes with decreased expression in the brain and, in some cases, the liver; **cluster K**, genes primarily down-regulated in the spleen; and **cluster L**, genes with decreased expression in the liver. See Supplementary Information for full data.

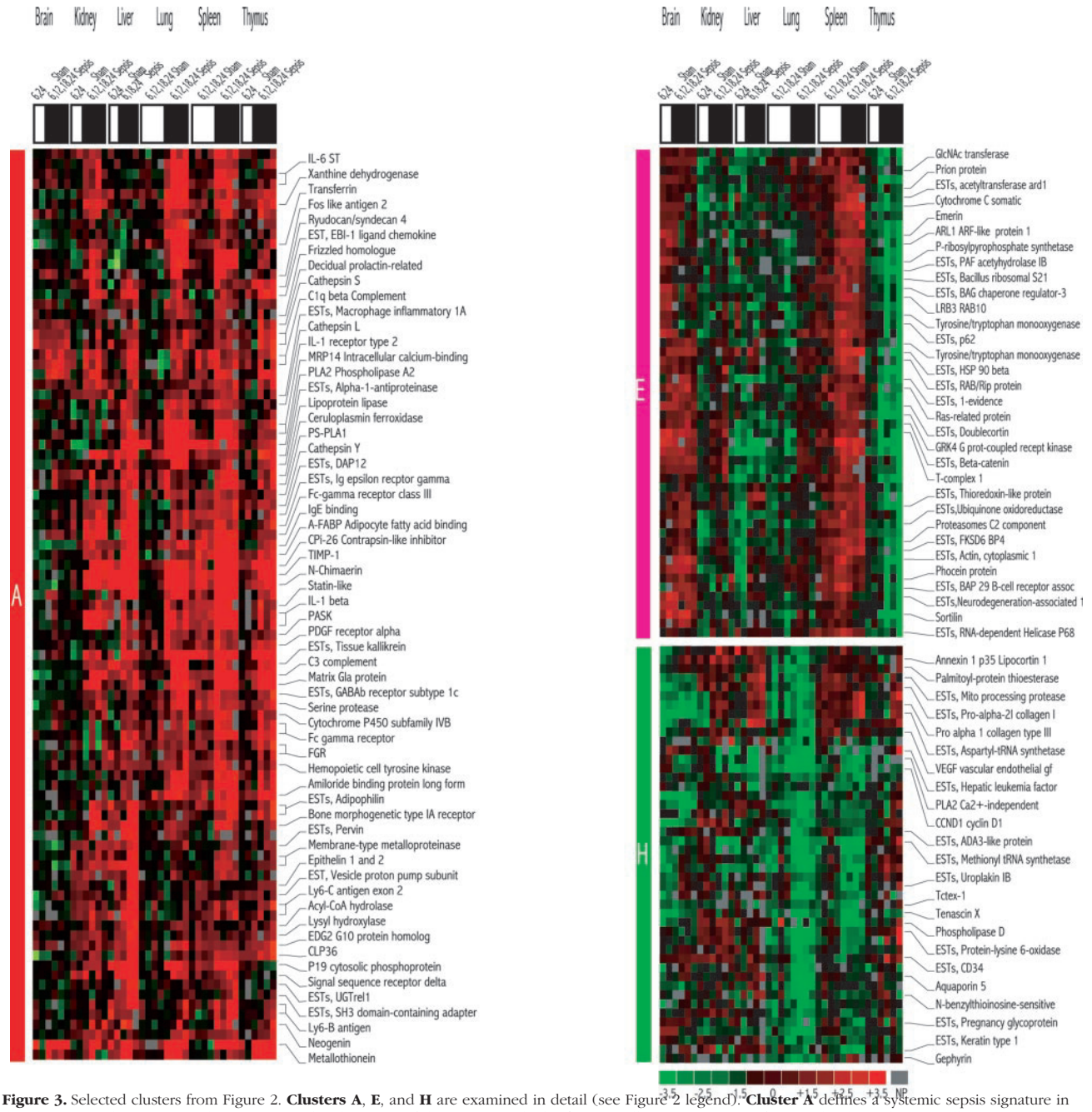

Figure 3. Selected clusters from Figure 2. **Clusters A**, **E**, and **H** are examined in detail (see Figure 2 legend). **Cluster A** defines a systemic sepsis signature in that this group depicts genes that are up-regulated in most of the tissues studied. **Cluster E** illustrates a group of genes that have diametrically opposite gene expression patterns in the spleen and in the thymus. **Cluster H** depicts genes that are down-regulated during the septic response (and in this case down-regulated in the lung and spleen). Unnamed genes are in most cases ESTs. See Supplementary Information for full data.

Validation of Selected Genes Identified by Microarray Analysis

Selected genes identified by our microarray screen were corroborated by Northern analysis of the six organs studied (Figure 4). For example, TIMP1 was found to be 3.8-, 4.4-, 3.1-, 3.7-, 1.2-, and 2.3-fold up-regulated by microarray in the CLP liver, lung, spleen, thymus, brain, and kidney, respectively (Figure 4A). Similarly by Northern analysis TIMP1 transcript was up-regulated in the same set of organs 12.3-, 2.5-, 4.6-, 17.8-, 2.3-, and 2.2-fold, respectively. Similar qualitative concordance between Northern and microarray analysis was achieved with other genes tested including N-chimaerin, PLA_2, and Ca^{2+}-independent PLA_2 (Figure 4, B and C).

Functional Analysis of Sepsis-Induced Gene Expression Patterns

We next assessed the data by examining functional groups of known, named genes (Figure 5). During the response to sepsis, bacterially derived lipopolysaccharide induces the appearance of a number of cytokines in

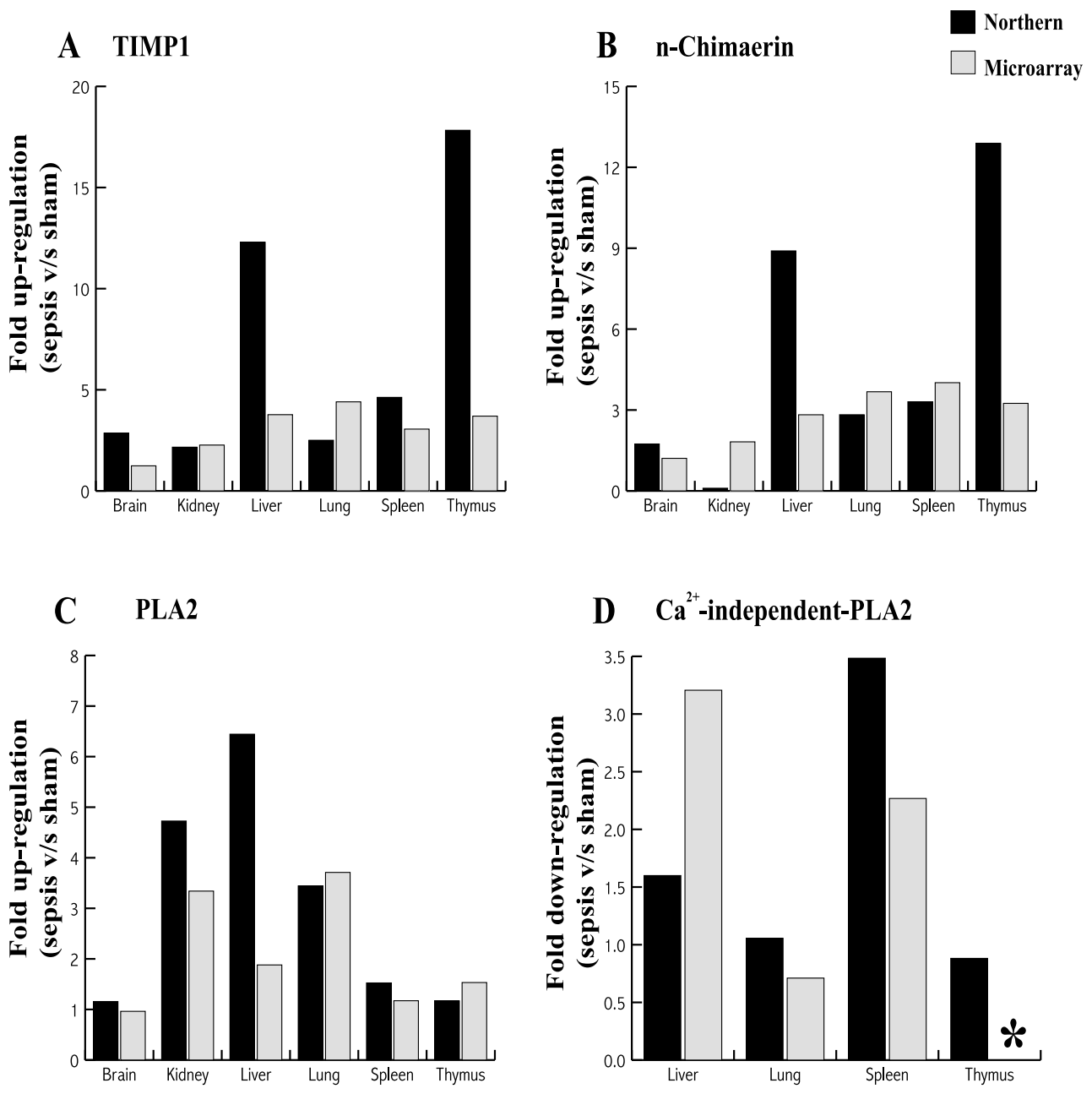

Figure 4. Validation of microarray results by Northern blot analysis. Fold up- or down-regulation of the respective transcript in CLP rats (24-hour time point) *versus* sham rats as determined by Northern blotting and cDNA microarray analysis. **Asterisk** refers to a microarray measurement that was not present. Ca^{2+}-independent PLA_2 transcript was not detected by Northern blot in brain or kidney tissue and was thus not reported. See Materials and Methods for details.

circulation that mediate the systemic response including TNF-α, IL-1, and IL-6. In the organs analyzed, increased TNF-α expression was only observed at the 6-hour time point in the CLP lung. This may be because of the higher concentrations of mononuclear phagocytes found in lung tissue or because of an increase in TNF-α that may occur before the 6-hour time point in CLP animals. As discussed earlier, it is interesting to note the coordinated gene expression of IL-1β and its negative regulator, IL-1RII (Figure 5). Coordinated up-regulation of these proteins is seen in the liver, lung, spleen, and thymus. Although IL-1β transcript is increased in the kidney, the corresponding transcript for IL-1RII is not. Differential regulation of either protein does not occur in the brain. Up-regulation of an inflammatory agonist (IL-1β) and its decoy receptor (IL-1RII) in the diverse organs tested likely represents a physiological mechanism to tightly regulate the inflammatory response. Although IL-6 is notably absent from our chip, IL-6 ST(gp130), which in conjunction with IL-6 receptor mediates IL-6 signaling, is up-regulated in the kidney, lung, and spleen. Interestingly, STAT3, which is a transcription factor integral to the gp130-signaling pathway, exhibited increased transcript expression in the liver, lung, and spleen. Thus, our data

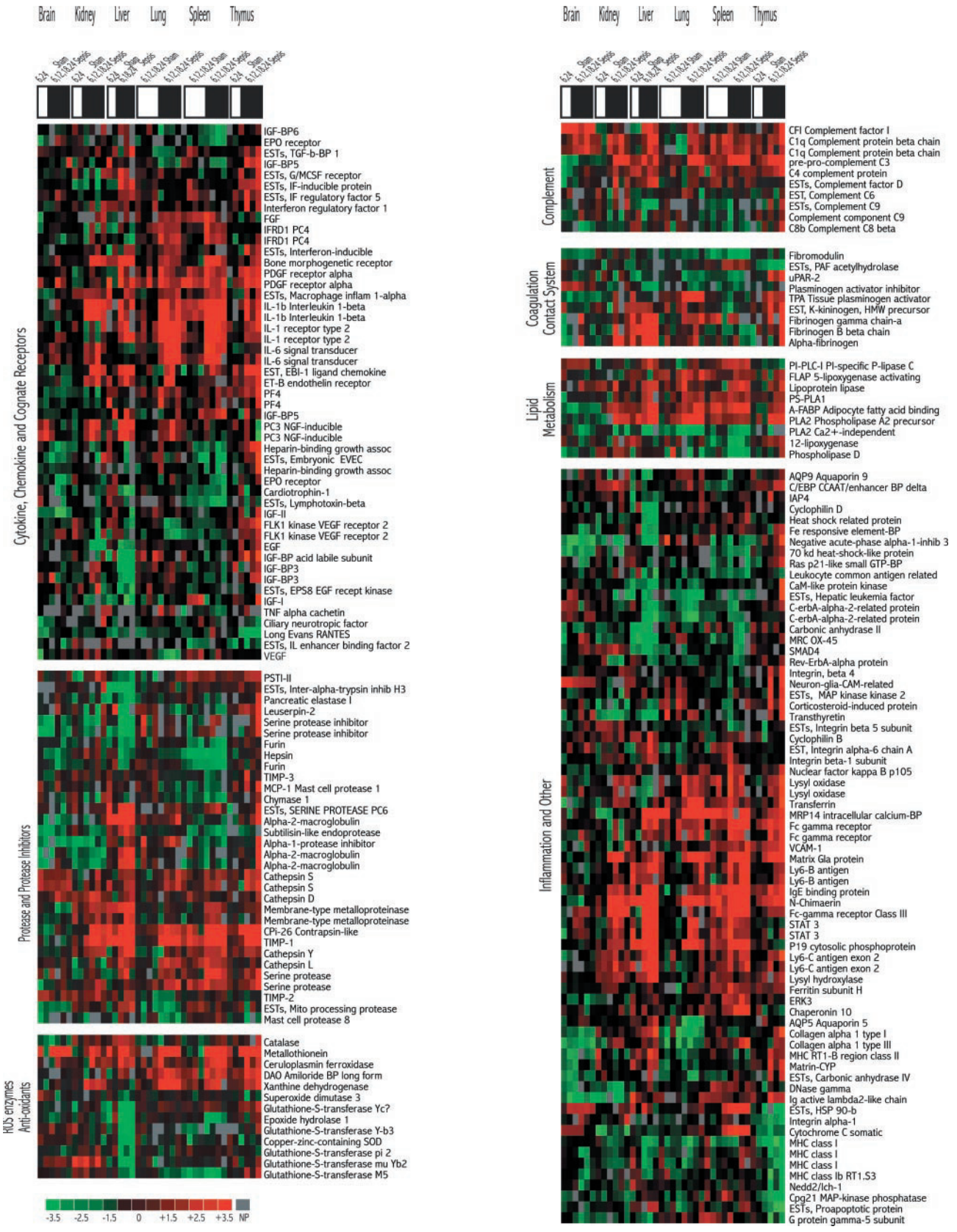

Figure 5. Functional clustering of sepsis-induced gene expression patterns. Selected inflammation-related genes that are differentially expressed in at least one organ of CLP rats are shown. The same convention for representing changes in transcript levels was used as in Figures 2 and 3. Genes are grouped into arbitrary functional categories. See text for details of discussion on individual genes.

suggest that several tissues mobilize downstream components of the IL-6 signaling pathway in response to sepsis and are presumably primed for activation by IL-6.

Cytokines involved in angiogenesis are also regulated during sepsis. FLK1 kinase [vascular endothelial growth factor (VEGF) receptor 2], along with its ligand VEGF, are specifically down-regulated in the lung. Although activation of the VEGF pathway induces vascular permeability, decreased transcript levels of VEGF and its receptor, as observed during the sepsis response, may prevent increases in vascular permeability and promote homeostasis during sepsis. Similarly, endothelin receptor, which mediates the vasoconstrictor activities of endothelin, was shown to have enhanced expression in the lung, possibly functioning as a mechanism to minimize fluid losses (into the lung).

Tissue injury during sepsis occurs by a variety of mechanisms including that mediated by reactive oxygen species and proteolytic enzymes. In the protease and anti-protease groups it is evident that the rat serine protease inhibitor, Cpi-26 (contrapsin-like inhibitor) and the metalloproteinase inhibitor, TIMP-1 are induced during sepsis in most of the tissues studied, suggesting that this functions as a mechanism for attenuating protease-mediated tissue damage. It is also reassuring to observe up-regulation of α-2 macroglobulin and α-2 anti-proteinase inhibitor in the liver, two classic acute-phase reactants. Other less characterized proteases and anti-proteases described in the rat are also differentially regulated during the septic response (Figure 5). Enzymes known to produce oxidative metabolites such as deamine oxidase (amiloride binding protein 1) and xanthine dehydrogenase are transcriptionally up-regulated during sepsis in most of the tissues studied (Figure 5). In addition to generating oxidants, deamine oxidase degrades histamine,[31] which has a central role in promoting allergic and acute inflammatory states. Xanthine dehydrogenase is produced by both epithelial cells and neutrophils and has been shown to be a major source of injurious reactive oxygen metabolites during tissue injury.[32,33] Similarly, proteins that have anti-oxidant effects such as metallothionein and ceruloplasmin are also up-regulated in a similar set of tissues. Both proteins have the ability to scavenge superoxide anion and may represent a defense mechanism against oxidant-mediated tissue injury.[26,34]

Activation of the complement system together with assembly of the membrane attack complex C5b-9 plays an important role in host defense and sepsis.[35] Components of the complement system such as C1q, C3, and to a lesser extent C4 are up-regulated in many tissues of the CLP animal (Figure 5). By contrast, C6, C8, and C9 do not display a similar gene expression pattern. Interestingly, the complement regulatory protein, Factor I (CFI), a serine protease that inactivates C3b and C4b,[36,37] is also up-regulated in many of the tissues tested. Concordant CFI up-regulation may serve as a defense mechanism against renegade activation of the complement system during the systemic response to sepsis.

Various proteins involved in arachidonic acid metabolism are induced in tissues of CLP rats. Cytosolic PLA_2, which is responsible for the release of arachidonic acid from phospholipid stores, is activated by submicromolar concentrations of Ca^{2+} and has recently been implicated in sepsis-induced lung injury.[21] Here we discover that PLA_2 is up-regulated at the gene expression level in many of the tissues we analyzed in the CLP rat including the lung (Figure 5). Interestingly, we observed coordinate decreases in the transcript levels of the Ca^{2+}-independent forms of PLA_2. This may represent another site of physiological regulatory control in the systemic inflammatory pathway. A number of other named genes (inflammation-related or otherwise) with twofold increases or decreases in transcripts relative to the respective control organs are also displayed in Figure 5 (Inflammation and Other). Notable examples of genes with increased expression in this group include $F_c\gamma$ receptor, MRP14, p19 cytosolic protein, and matrix G1a protein. Similarly, genes with decreased expression, in selected organs of this arbitrary grouping, include c-*erb*-A-α-2-related protein, negative acute phase apha-1 protein, and MHC class I proteins.

Discussion

A daunting clinical challenge has been the successful treatment of humans with sepsis despite the availability of powerful, broad-spectrum antibiotics. In surgical and medical intensive care units, sepsis often leads to functional impairment of the lungs, resulting in acute lung injury or development of the adult respiratory distress syndrome. Other frequent complications include hepatic and renal failure, the triad commonly being referred to as "multiorgan failure syndrome."[38–41] Although this pattern of organ failure is well-known clinically, its pathogenesis is poorly understood. It has been postulated that sepsis after blunt trauma, penetrating injuries, ischemia, and various other clinical conditions, especially when the abdomen is involved, may be the result of gram-negative bacteria translocating into the gut wall from mucosal surfaces, appearing subsequently in adjacent lymphatics and/or the blood stream,[42] and may cause systemic changes that first present as hyperdynamic events (eg, increased cardiac output, tachypnea, hyperthermia, leukocytosis, hypocapnia, hypermetabolism, and so forth) followed, as sepsis proceeds, by development of the hypodynamic state (eg, reduced cardiac output, decreased peripheral vascular resistance, hypothermia, hypercapnia, reduced PaO_2, and so forth).[43,44] Sepsis is associated with a systemic inflammatory response syndrome that is characterized by the appearance in plasma of cytokines (eg, IL-6, TNF-α, IL-1), suggesting that regulation of the inflammatory response has been compromised.[4,45] There is evidence of complement activation, as reflected by falling levels in plasma of the hemolytic activity of complement (CH50) and the appearance in plasma of complement activation products such as C3a and C5a, together with the membrane attack complex, C5b-9.[45,46] Although the complement system (especially complement activation products, C3b, iC3b, and C5b-9) is a vital defensive system against invasion by bacteria,

there also exists the possibility that excessive complement activation can result in compromised host defenses.

Several overriding themes emerge from our multiorgan gene expression study of sepsis. Microbial infection and the associated systemic sepsis response triggers a massive activation of transcriptional programs in the individual organs/tissues of a whole organism. Not only is an array of genes induced during sepsis but an equally interesting set of genes is repressed. One of the most intriguing aspects of this study is the comparison of gene expression patterns of different tissues to a systemic stimulus. Although there are subsets of genes that share similar expression patterns in many organs (with the brain being a frequent exception), each organ has a distinctive molecular response to systemic inflammation. The blood-brain barrier may be responsible for the apparent lack of the common sepsis signature in the brain. There are also interesting associations between organs. For example, a distinct set of genes is up-regulated in the thymus and coordinately down-regulated in the spleen (Figure 3). Does this have to do with different sepsis responses by thymocytes *versus* B and T lymphocytes? Further experimentation will be needed to decipher this interaction between the thymus and the spleen. Finally, it is also quite evident that a specific set of genes is differentially expressed in an organ-specific manner (Figure 3). The molecular basis for these tissue-common and tissue-specific responses remains to be discovered.

Genes with proinflammatory effects were often balanced by genes with anti-inflammatory effects illustrating the regulatory controls embedded in this complex pathway. Examples of this Yin-Yang gene expression include: 1) IL-1β and its decoy receptor; 2) reactive oxygen metabolite generating enzymes and superoxide destroying enzymes; 3) complement components (C1q, C3, C4) and an inactivator of complement components (CFI); and 4) induction of Ca^{2+}-dependent PLA_2 and coordinated repression of Ca^{2+}-independent PLA_2.

Differential gene expression was observed in proteins responsible for preventing tissue injury and promoting homeostasis including anti-proteases (TIMP-1, Cpi-26), oxidant neutralizing enzymes (metallothionein), cytokine decoy receptors (IL-1RII), and tissue/vascular permeability factors (aquaporin 5, VEGF). Genes previously implicated in the inflammatory process were studied in the context of sepsis at a multiorgan level. Likewise, genes not known to be involved in sepsis were also characterized. Numerous ESTs were assigned by gene expression patterns to the sepsis clusters described in Figure 2 (guilt by association). Further characterization of the sepsis-induced gene expression profiles obtained here may identify novel sepsis biomarkers and shed light into the etiology of multiple organ failure, an often-fatal complication of systemic inflammation. By profiling gene expression at a multiorgan level in an animal model of systemic inflammation, it will soon be possible to determine potential anti-inflammatory effects of emerging therapeutics.

Supplementary Information

Sepsis profiling datasets (DNA microarray datasets) will be available at the author's website: http://chinnaiyan.path.med.umich.edu/.

Acknowledgments

We thank Anjana Menon and Robin Kunkel for assistance in preparing the rat cDNA clones and preparation of figures, respectively.

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