Expression profiling analysis of the metabolic and inflammatory changes following burn injury in rats

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Submitted 7 November 2003; accepted in final form 23 April 2004

Vemula, M., F. Berthiaume, A. Jayaraman, and M. L. Yarmush. Expression profiling analysis of the metabolic and inflammatory changes following burn injury in rats. *Physiol Genomics* 18: 87–98, 2004. First published April 27, 2004; 10.1152/physiolgenomics.00189.2003.—Burn injury initiates an inflammatory response as part of the healing process that is associated with extensive metabolic adjustments. While most studies have focused on understanding these changes from a biochemical perspective, not much work has been done to characterize these processes at the gene expression level. As a first step, we have comprehensively analyzed changes in gene expression in rat livers during the first 24 h after burn injury using Affymetrix GeneChips, which showed 339 genes to be differentially expressed at a statistical significance of $P < 0.05$ and changed at least twofold. Functional classification based on gene ontology terms indicated that two categories, metabolism (28%) and inflammation (14%), accounted for nearly 42%. Detailed analysis of the metabolism group of genes indicated that fatty acid (FA) and triglyceride (TG) biosynthesis in the liver were unchanged, whereas TG utilization, FA import, and β-oxidation increased after burn injury. The increased FA pools after burn injury appear to serve as substrates for ATP production. Following burn injury, the cholesterol biosynthetic pathway was suppressed while cholesterol was increasingly imported and converted into bile acids. The inflammatory genes that were altered included several classic acute phase response markers, as well as genes involved in the complement, kinin, clotting, and fibrinolytic protein systems. These temporally coordinated changes in gene expression were also corroborated by biochemical measurements for FA, TG, cholesterol, and ATP. Together, these data indicate that FA are increasingly imported and oxidized in the liver to meet the enhanced energy demands arising from an inflammatory response during the first 24 h after burn injury.

liver; burn mRNA; fatty acids; cholesterol; microarrays

The body’s response to burn injury is characterized by extensive changes in several physiological processes including metabolism and inflammation. Metabolic responses include an increase in the basal metabolic rate, nitrogen metabolism, and proteolysis (13, 27). The increase in energy expenditure following burn injury (27) has been attributed in part to the metabolic processes such as gluconeogenesis, ureagenesis, futile cycles [fatty acid (FA) synthesis, FA breakdown, and Cori cycle], and the necessity to compensate for the increased loss of body heat through injured skin (27). Burn injury also results in dramatic changes in the circulating levels of plasma proteins (5). Since almost all of the plasma proteins are synthesized in the liver, an increase in plasma protein levels following burn injury also causes increased energy expenditure (38) and affects liver performance and function.

In this study, we hypothesize that the increase in energy expenditure results in part due to significant changes in the expression of genes involved in pathways for substrate and energy metabolism. In general, metabolic changes following burn injury have been studied using biochemical and physiological techniques (28, 45). Several laboratories have undertaken organ-specific studies (10, 16, 24, 36, 40) that involve administration of a thermal injury to an animal, followed by perfusion of the organ for determining net rates of production or uptake of various metabolites (28, 45). This systems approach has been used to describe changes in metabolite levels and establish a metabolic basis for the liver response to burn injury (10, 16, 24, 36, 40). However, these techniques are limited by the number of metabolic reactions that can be studied simultaneously and hence lack the ability to comprehensively describe a disease state. The metabolic alterations are only one component of the overall liver response, and other aspects such as changes in liver gene expression following burn injury have not yet been fully characterized. Moreover, understanding the genetic basis for the disease state may provide clues for the development of improved therapeutics (17). The recent advances in antisense technology to silence genes and the growing number of associations between the single nucleotide polymorphisms and a patient’s disposal to a disease state are additional motivations to study the changes in gene expression (31). The need for obtaining both gene expression profiles and biochemical metabolite data for a comprehensive understanding of metabolic states forms the fundamental basis of the approach outlined in this paper.

Here we report the changes occurring in rat livers upon burn injury, as analyzed using both Affymetrix GeneChips and biochemical metabolite measurements. Nearly 42% of the genes whose expression was altered upon burn injury were involved in either inflammatory or metabolic processes. Our data indicate an increase in energy production in the liver during the first 24 h after burn injury as well as in the reverse transport of cholesterol leading to the production of bile acids. These temporally coordinated changes were also corroborated with biochemical measurements for FA, triglycerides (TG), cholesterol, bile acids, and ATP. Our results also show the activation of the four protein systems, i.e., the complement, kinin, clotting, and fibrinolytic protein systems, as part of the inflammatory response to burn injury. Together, these data indicate that FA serve as the energy sources in the liver during the first 24 h following burn injury.
MATERIALS AND METHODS

In Vivo Experiments

Animal experiments were performed with male Sprague-Dawley rats (Charles River Laboratories, Boston, MA) weighing 150–200 g. All experimental protocols used in this study were approved by the Subcommittee on Research Animal Care, Massachusetts General Hospital. Rats were individually housed in a temperature-controlled (25°C) and light-controlled room (12:12-h light-dark cycle) and adjusted to their new surroundings for at least 5 days prior to the experiment. Water and rat chow were provided ad libitum to the animals. On the day of the treatment, the animals were randomly divided into two groups: burned and sham burned. The burn injury consisted of a full-skin-thickness scald burn of the dorsum, calculated to be ~20% of the rat’s total body surface area (TBSA), induced by immersing the designated area in boiling water for 10 s (45). Rats were resuscitated with an intraperitoneal injection of sterile saline solution (1.5 ml/kg body wt) immediately after burn. A study by Herndon et al. (22) found no difference in the feeding patterns between the sham-treated and burn-injured animals for this model. The mortality rate of this treatment was negligible. At each time point (1, 4, 8, and 24 h), three animals belonging to each group were killed, and the liver and serum were collected and stored at −80°C, after the animals were infected with 20% TBSA burn injury. Sham-burn rats (n = 3) considered as the control or 0 h time point were treated identically except that they were immersed into a 37°C water bath and immediately killed to collect the liver. Sham samples were not collected for all time points (1, 4, 8, and 24 h), because prior data from our laboratory (29) showed that time had no effect on the metabolite fluxes after sham treatment.

Preparation of Labeled Target and Hybridization

For each time point (0, 1, 4, 8, and 24 h), total RNA was isolated from ~50 mg of pooled liver tissue using the Nucleospin II RNA isolation kit from BD Biosciences (Palo Alto, CA). Briefly, the tissue was homogenized in the presence of buffer RA1 (supplied by the manufacturer) and β-mercaptoethanol. To the clear homogenate, 70% ethanol was added and loaded onto the column to retain the RNA on the membrane. After DNase I treatment, the RNA was eluted into 30 μl of RNase-free water. Then, 15 μg of biotinylated cRNA (prepared from 20 μg of total RNA using protocols provided by Affymetrix) was used to hybridize two identical rat U34A GeneChips that had 8,799 genes represented on each GeneChip (Affymetrix). The GeneChips were washed, scanned, and analyzed using Affymetrix GeneChip MAS ver. 5.0 software.

Data Analysis

All GeneChips were scaled to a target intensity of 500 to account for differences between the replicate GeneChips and their hybridization efficiencies using Affymetrix GeneChip MAS ver. 5.0 software. The entire dataset is available at the NCBI Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/) with the accession number GSE802. Three filters were serially used to obtain the list of annotated genes that demonstrated differential expression in intensity between sham and burn-injured animals. In the first filter, only genes that were flagged as “present” by the MAS ver. 5.0 analysis software in at least one of the time points in all replicate GeneChips were considered for further analysis. This step eliminated all genes for which the expression data was not reproducible between the replicate GeneChips. The genes that passed this criterion were subjected to a second filter where ANOVA was performed to test each gene independently for a statistical difference in expression between any one time point (1, 4, 8, or 24 h). The output of the analysis is the probability (P value) that a difference in expression can be observed by chance i.e., probability of getting a false positive. Although the occurrence of false positives can be controlled by choosing a higher significance level (0.01 or 0.001), it also concomitantly increases the false negatives. Therefore, in this study we have chosen to work with ANOVA P value cutoff 0.05. To reduce the occurrence of the false positives, we have used the false discovery rate (FDR) method (33), which adjusts the ANOVA P value (cutoff 0.05) while taking the dependencies between the genes into consideration. The 695 genes that demonstrated a significant ANOVA P value were then selected, and their expression values were averaged. The third filter selected 339 genes that were either upregulated or downregulated by at least twofold between any one time point (1, 4, 8, or 24 h) and the 0 h time point (Sham control). To determine the temporal profiles in the data, gene expression values were hierarchically clustered using the dChip software (http://www.dChip.org). The expression values for a gene across all samples were standardized by setting the mean to 0 and standard deviation to 1. The software then builds a hierarchical cluster tree based on centroid-linkage method using the Pearson correlation coefficient as the distance metric. A set of genes were assigned to cluster groups empirically based on visual inspection of their temporal expression patterns.

Biochemical Assays

TG levels in liver extracts were measured using a commercially available kit (Sigma, St. Louis, MO), based on the release of glycerol from TG by lipoprotein lipase. Replacing the LPL in the TG kit with water permitted the quantification of free glycerol in the supernatant. Free FA were measured using a commercial kit from Roche Molecular Biochemicals (Indianapolis, IN) based on the release of hydrogen peroxide in the presence of acyl-CoA synthetase and acyl-CoA oxidase that catalyze the conversion of FA into enoyl-CoA. Cholesterol assays were performed using a commercial kit from Equal Diagnostics (Exton, PA) based on the enzymatic conversion of cholesterol ester to cholesterol and FA by cholesterol esterase followed by the reaction of free cholesterol with cholesterol oxidase. ATP levels were measured using the ATP Bioluminescence Assay Kit HSI from Roche Molecular Biochemicals, which uses the ATP dependency of the light emitting luciferase-catalyzed oxidation of luciferin.

RESULTS

Male Sprague-Dawley rats were subjected to a 20% TBSA scald-burn injury, and liver tissue was collected at 1, 4, 8, or 24 h after burn injury. Changes in the expression of liver genes following burn injury were monitored using high-density Affymetrix GeneChips, and 339 annotated genes of 8,799 represented on the array were selected for further analysis. In addition, two other genes, low-density lipoprotein receptor (Ldlr) and scavenger-receptor BI (SR-BI), whose expression levels were altered at least by twofold but did not pass the ANOVA filter, were included in the data set to give a better perspective of the reverse cholesterol transport pathway that was altered by burn injury, which is explained in detail later. The expression intensities of all 8,799 genes represented on the GeneChip mostly varied in the range of 10–10,000, whereas the 341 selected genes fell into the higher end of the expression range (100–10,000) (Fig. 1). These 341 genes were sorted into 11 functional categories based on the Gene Ontology Consortium classification (http://www.geneontology.org), UniGene and Swiss-Prot databases, and scientific literature available at PubMed (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi) (Fig. 2). Broadly, these categories reflected changes that are typical following burn injury, including metabolic adjustments and inflammation. The metabolism (28%) and inflammation (14%) categories alone accounted for 42% of the genes that were selected as differentially expressed in response to burn injury.
Hierarchical clustering was used to characterize the temporal expression pattern of the 145 genes in metabolism and the inflammation categories that were altered by burn injury (Fig. 3). These genes were sorted into 11 gene expression clusters based on the temporal expression profiles such that each cluster was characterized by a distinct pattern. Three dominant temporal patterns of expression were evident from the clustered data including a monotonic change in expression, a transient change in expression, and biphasic alterations or repeated transient changes in gene expression. For example, the expression of genes in clusters 1, 3, and 4 decreased over the duration of the experiment (Fig. 3), whereas genes in clusters 2, 8, and 9 transiently increased in expression following burn injury. Similarly, genes in clusters 7 and 11 initially increased followed by a rapid decrease and another increase in gene expression levels. These results indicated coordinated changes in
the expression of several classes of liver genes following burn injury. Below we describe the expression patterns of a subset of genes which can be divided into two main groups: metabolism and inflammation.

Changes in Metabolic Processes Following Burn Injury

Burn injury altered the expression of genes in several metabolic pathways including TG and FA breakdown, glucose metabolism, ATP production, cholesterol transport, and bile acid production.

Triglyceride and fatty acid metabolism. Gene expression profiling of liver tissue after burn injury showed decreased biosynthesis and increased utilization of FA and TG. The decrease in expression of genes involved in FA and TG biosynthesis, although evident (see the Supplemental Material for this article, which is available at the Physiological Genomics web site), was not significant enough to meet the criteria outlined above for selecting differentially expressed genes. In addition, the expression of genes involved in the synthesis of mono-unsaturated FA and the precursor for FA biosynthesis were both significantly reduced as seen with a 3.5-fold decrease in the levels of stearoyl-CoA desaturase (Scd2) and the 2.5-fold decrease in the expression of ATP-citrate lyase (Acly) (Fig. 4B). However, both Scd2 and Acly did show a marginal increase of 1.3- to 1.4-fold in their expression and could result in a slight upregulation in their respective activities. The expression of several genes involved in the pathways for TG and FA utilization also showed a transient increase (>2-fold). A summary of these changes in expression for genes involved in TG breakdown, FA trafficking, carnitine-mediated transport, and mitochondrial/peroxisomal FA oxidation is shown in Fig. 4A.

The levels of TG and FA in the liver after burn injury were also measured using standard biochemical techniques. No significant change in TG levels was observed until 24 h postinjury (Fig. 5A); however, FA levels increased by 20–30% at 1 h and 4 h (Fig. 5B). By 24 h after burn injury, the levels of both TG and FA decreased by 37% and 12%, respectively, compared with the sham controls (Fig. 5, A and B). Taken together with the gene expression data, these results suggest that burn injury induced 1) decreased biosynthesis of TG and FA, 2) increased utilization of TG, and 3) increased β-oxidation of FA in the liver after burn injury.

ATP synthesis. Acetyl-CoA generated through FA oxidation can serve as substrates for ATP production through TCA cycle and electron transport pathway. The gene expression data, however, showed that none of the genes involved in electron transport chain and ATP synthesis pathways was altered significantly enough to meet the filtering criteria described above. Therefore, based on the gene expression data alone, it was not

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1 The Supplementary Material for this article is available online at http://physiolgenomics.physiology.org/cgi/content/full/00189.2003/DC1.
possible to determine the fate of the oxidized FA in the liver. However, biochemical measurements of ATP levels showed an 18% increase by 8 h compared with the control, followed by a decrease to 25% below the initial value at 24 h (Fig. 6). This result, in combination with the gene expression data, suggested that some of the oxidized FA were being converted into ATP.

Glucose metabolism. The gene expression data showed that expression of two key gluconeogenic enzymes, phosphoenolpyruvate carboxykinase (Pepck) and glucose-6-phosphatase (G6pc), decreased by 2.6-fold and 5.8-fold, respectively, within 24 h after burn injury (see the Supplementary Material). Moreover, the expression of the key glycolysis enzyme, pyruvate kinase (Pkl), decreased by 3.3-fold at 8 h postburn, which indicated that glucose was neither being synthesized nor utilized as an energy source during the first 24 h after burn injury.

Reverse cholesterol transport and bile acid production. Burn injury resulted in the downregulation of genes involved in the liver cholesterol biosynthesis pathway as well as an upregulation of genes responsible for hepatic uptake of cholesterol from the plasma and its conversion to bile acids (Fig. 7, A).

Fig. 4. Alterations in triglyceride (TG) and fatty acid (FA) metabolism after burn injury. A: the pathway for TG metabolism and FA oxidation in the liver is shown along with the genes that were significantly altered by burn injury. An increase (↑) or a decrease (↓) in their expression pattern is shown in parenthesis, and these include genes involved in TG breakdown (Lipc, hepatic lipase); FA transport (Fabp, fatty acid binding protein); carnitine-mediated shuttle into mitochondrion or peroxisomes (Crot, carnitine palmitoyltransferase; Cpt1a, carnitine octanoyltransferase); and FA oxidation (Acaal1, peroxisomal 3-ketoacyl-CoA thiolase; Dc1, 3,2 trans-enoyl-CoA isomerase; Eci, 5,3,62-enoyl-CoA isomerase; and Cyp4a3, cytochrome P450 1A3). Pathways that were suppressed are represented by the symbol “X”. B: the genes involved in TG and FA metabolism were clustered using dChip software as explained in MATERIALS AND METHODS. The average expression pattern for genes clustered together is shown adjacent to the group. In every case examined, when a gene was represented by more than one probe element, the multiple representations were seen to have identical or very similar expression profiles.
The levels of genes in the committed cholesterol biosynthesis steps, HMG-CoA synthase (Hmgcs1) and squalene synthase (Fdft1), decreased 5.2-fold and 3.0-fold, respectively, as early as 4 h after burn injury. The expression of the specific receptor for importing HDL-cholesterol esters (HDL-c) from extrahepatic tissues, scavenger receptor B1 (SR-B1) (1, 25), gradually increased to 2.2-fold by 4 h after burn compared with the control (Fig. 7B). On the other hand, the receptor solely responsible for LDL cholesterol uptake into the liver (Ldlr) (7) decreased by 3.4-fold within 4 h after burn injury (Fig. 7B). The expression of cholesterol esterase (Cel) which is involved in the breakdown of the imported cholesterol in the liver also increased 5-fold within 1 h of burn injury.

Several genes involved in bile acid production, including cholesterol-7-/H9251-hydroxylase (Cyp7a1, 1.9-fold), alcohol dehydrogenase (Adh3, 2-fold), alcohol dehydrogenase (Adh1a, 2.2-fold), and aldehyde dehydrogenase (Aldh1a4, 2.6-fold), increased maximally at 8 h or 24 h following burn injury. The expression of the transporter for taurine (Slc6a6), a molecule that is conjugated to the primary bile acids to produce secondary bile acids, also increased transiently (3.1-fold) at 4 h after burn injury. On the other hand, genes involved in the degradation of bile acids through sulfation (sulfotransferases Sth2, Sult1c2; α-methylacyl-CoA racemase, Amacr) were down-regulated 2- to 4-fold within the first hour of burn injury.

Biochemical measurements of total cholesterol levels in the liver also showed a 70% increase during the first 4 h after burn injury; however, they gradually decreased to 30% below the initial value by 24 h (Fig. 7C). Together, the gene expression and biochemical data showed opposite trends for cholesterol biosynthesis during the first 4 h following burn injury. However, data from both measurements indicated increased conversion of cholesterol into bile acids in the liver by 24 h postinjury.

Changes in inflammatory responses following burn injury. Several genes involved in the innate and acquired immune response (classified as inflammation-related in Fig. 8) demonstrated marked alterations in expression following burn injury. The expression of proinflammatory molecules such as chemokine Gro (19.8-fold) increased in the liver at 4 h after burn injury.
injury. This increase was also mirrored by a transient increase in the expression of the TNF-α receptor (Tnfrsf1a) and the soluble IL-1 receptor type I (Il1r1) at 4 h, with the mRNA level of the TNF-α receptor and Gro increasing again at 24 h.

The expression of several classic inflammatory acute phase response genes such as serum amyloid (Saa2), α2-macroglobulin (A2m), α-fibrinogen (Fga), α1-acid glycoprotein (Orm1), and lipocalin (Lcn2) were all upregulated in the liver following...
burn injury (Fig. 8). The maximal changes in the expression of these genes varied from 3- to 21-fold, and although no single dominant kinetic pattern was evident, a majority of the changes occurred at either 8 h or 24 h after burn injury. Other proteins that were upregulated after burn injury are part of the four protein systems activated in response to the injury. These include acute phase response markers (serum amyloid, Saa2; α2-macroglobulin, A2m; α-fibrinogen, Fga; α1-anti-trypsin, Orm1; and lipocalin, Lcn2), opsonins (complement factor, C4bpa), vasodilators (C4a), membrane attack complex (complement, C9), clotting factors (α-fibrinogen, Fga; plasminogen, Plg), protease inhibitors (Spin2c, 4.3-fold; A2m), synthesis of bradykinin (kinogen, major acute phase α1 protein, Kg1). The category also included genes involved in antigen processing and presentation (proteasome subunit RC1, Psmb8; MHC class I and II; cyclic protein 2, Cp-2), and pattern recognition receptors (lipopolysaccharide binding protein, Lbp).

The inflammation category also included several genes that are involved in the antigen processing and presentation (Psmb8, MHC class I, and II, Cp-2) and pattern recognition receptors (Lbp) that defend the host against invading pathogens. All of the antigen processing and presentation genes, except Cp-2, predominantly increased at 8 h. Cp-2 showed an increase at 4 and 24 h after burn injury.

**DISCUSSION**

The objective of this study was to characterize the changes in gene expression patterns during the first 24 h following burn injury. Although gene expression profiling is being increasingly used to study disease states (6, 32, 41, 23), it has only recently been applied to the study of burn injury and trauma (4, 11, 30, 39). However, to our knowledge, this is the first report characterizing gene expression changes in the liver after burn injury using microarrays. Although microarrays allow the estimation of mRNA abundance for thousands of genes simultaneously, the statistical limitations and the inability to interpret the enzyme activity based on mRNA levels must be considered. For instance, the P value associated with the ANOVA statistical test allows 5% of the genes selected as significantly altered to be false positives. Additional procedures such as FDR correction do not fully eliminate the false positives, and hence, some genes will appear as positives by virtue of their presence on the array. Moreover, gene expression data alone do not fully explain complex physiological states, as changes in transcript levels could be just one factor involved in invoking a specific response. Microarrays only measure the amount of mRNA expressed by a gene and do not provide information on the posttranslational modifications and half-life of the enzyme synthesized from the mRNA. In case of the hepatic response to burn injury, it has been well documented (2, 8, 9, 28, 29, 37, 46) that changes in metabolite levels independent of the gene expression also play an important part in determining the ultimate physiological status. Therefore, we have used both gene expression profiling and biochemical metabolite measurements to provide a more complete description of changes initiated in the liver after burn injury. The utility of this combined approach is evident as the biochemical techniques...
not only support the inferences from the gene expression data, but also provide additional information on specific processes occurring in the liver after burn injury. For example, the increase in the expression levels for genes involved in FA oxidation observed at 8–24 h after burn injury helped in interpreting and corroborating the biochemical measurement of a decrease in the free FA levels.

The gene expression and biochemical data presented here together depict a picture of temporally coordinated changes in the liver upon burn injury (Fig. 9). The early part (0–4 h) of the response is characterized by increased import of energy resources as FA and cholesterol esters, whereas the latter part (8–24 h) shows enhanced breakdown and utilization of the energy sources possibly for the synthesis of acute phase proteins. Our data clearly indicate that fat metabolism is altered in the liver during the first 24 h after burn injury, showing an increase in the levels of FA during the first 4 h and a decrease beyond 8 h. The increase in FA levels can be due to increased

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Fig. 9. Proposed network of metabolic changes in the liver following burn injury. The clear box enclosing metabolites represents the liver. The significant changes that occur at different time points (1, 4, 8, and 24 h) following burn injury are shown as bold arrows, with green representing interpretations based on gene expression data, red representing interpretations based on biochemical data, and orange representing the interpretations based on both gene expression and biochemical data. The metabolites represented include very low-density lipoprotein (VLDL), cholesterol esters (CE), cholesterol (C), A-CoA (acetyl-CoA), bile acids (BA), amino acids (AA), and acute phase protein synthesis (APP synthesis).
biosynthesis or increased import. However, the expression levels of *Acly* only increased marginally (1.3-fold), and given that the enzyme has a half-life of 30 s (42), the synthesis of the precursor for FA biosynthesis can only be limited. Moreover, there was no increase in the expression of genes involved in FA biosynthesis, suggesting that the observed increases in biochemical measurements of FA are due to their import from extrahepatic tissues immediately (0–4 h) after burn injury. However, the decrease in FA levels at 8 and 24 h clearly suggested their consumption through FA oxidation. The decrease in TG by 24 h postburn could be due to either their increased export into the bloodstream or enhanced hydrolysis to produce FA. The export of TG into the bloodstream after burn injury is supported by the data on enhanced expression of apo-AI, a major constituent of VLDL particles along with the TG, as well as prior reports on increased levels of TG in the blood plasma after burn injury (12, 21) (Fig. 9).

Our observations on the decrease in FA biosynthesis and increase in FA oxidation during the first 24 h are in direct contrast to the changes in these processes by 96 h after burn injury (data not shown). This suggests a picture where FA are utilized in the liver during the first 24 h after burn injury but not at 96 h. Several reports (3, 18, 20, 44) have also suggested that glutamine and alanine derived from skeletal muscle are converted into glucose, which serves as the primary source of energy during sustained chronic inflammation. Although we do not observe an increase in the expression of transporters utilized for the uptake of glutamine and alanine into the liver and also the genes involved in gluconeogenesis during the first 24 h, preliminary gene expression data from our laboratory at 96 h postburn shows a 30-fold increase in the expression of the glutamine transporter in the liver (data not shown). Therefore, it is possible that different metabolites (such as FA and gluconeogenic amino acids) serve as the main source of energy in the liver at different times following burn injury, with FA being utilized and depleted prior to skeletal muscle breakdown and the import of amino acids into the liver when inflammatory conditions persist.

The lack of changes in the expression of genes involved in the electron transport chain and ATP synthesis, coupled with the initial increase in ATP levels as shown by biochemical assays, suggests that these genes are primarily regulated at the level of protein synthesis and membrane potential as reported earlier (26). The increase in ATP levels by 8 h indicates at least some of the FA oxidized to ATP, whereas the subsequent decrease at 24 h could be a result of either a decrease in ATP synthesis or an increase in ATP consumption. Since this decrease in ATP levels also coincides with the onset of increased acute phase protein synthesis (Figs. 8 and 9), it is possible that some of the ATP produced from FA oxidation is being utilized to meet the energy demand arising from increased acute phase protein synthesis.

An analysis of the cholesterol metabolic pathway also suggested that the increase in cholesterol levels soon after burn injury (0–4 h) is likely due to its import from extrahepatic tissues as the expression of genes in cholesterol biosynthesis was suppressed in the liver. Moreover, the cholesterol esters imported into the liver are likely associated with HDL and not LDL, as inferred from the increase in the expression of the HDL receptor *SR-B1* and the decrease in the expression of LDL-c receptor. The import of cholesterol esters (Fig. 9) is also evident from the increase in the expression of heparan sulfate proteoglycan (*Hspg*) mRNA during the first 4 h after burn injury, which has been shown to be involved in the import of TGs and HDL cholesterol esters into the liver (34). This observation is in good agreement with prior reports (12, 35) that have shown a decrease in the level of HDL cholesterol in the plasma after burn injury. The increased conversion of cholesterol into bile acids combined with the decreased breakdown of the bile acids could be a potential mechanism for solubilizing FAs and increasing their catabolism in the gut through the formation of mixed micelles.

The genes categorized as “inflammation” included several markers that are part of the acute phase response whose concentration is primarily dependent upon the production rate in the liver (5, 19). Although, the precise function for many of the acute phase proteins is not known, a number of them are multifunctional and contribute to both onset and resolution of inflammation. As part of the inflammatory response to burn injury, four protein systems were activated: the clotting, fibrinolytic, kinin, and the complement system. The clotting system, which yields fibrin for formation of blood clots to limit bleeding and stop the spread of infection, was promoted by increasing the expression of α-fibrinogen (*Fga*). Also, the fibrinolytic system that breaks down the clots was suppressed (as seen with the decrease in expression of plasminogen) during the first 24 h, suggesting that clotting was being favored. However, hypercoagulability or excessive clotting that occurs 2–3 h following scalding (48) appears to be controlled by increased expression of protease inhibitors (*Spin2c* and *A2m*) by 8 h. The kinin system responsible for the synthesis of bradykinin, a potent vasodilator and chemotactic, appeared to be activated as seen with an increase in the expression of kininogen (*Kng*) and major acute phase α-1 protein (*Kng*). Burn injury also increased the expression of complement proteins that include opsonins (*C4bpa*), vasodilators (*C4a*), and the membrane attack complex (*C9*). An intricate relationship exists between the various systems involved in the inflammatory response. The kinin, complement, and antigen processing and presentation systems are considered more important to host defense, and the clotting and fibrinolytic systems more important as homeostatic mechanisms. However, the different systems work together toward common goals, demarcation of necrotic tissue, control of infection, and wound healing.

The choice of experimental model as well as the nature of the insult can also determine the physiological processes impacted by the injury. For example, striking differences in the expression of inflammation-related genes are observed between our rat burn injury model and a mouse infection model used by Yoo and Desiderio (47). Although genes involved in clotting, increased blood flow, or protease inhibition were significantly altered in our study, the expression of these genes was not altered in the infection model (47). However, the two model systems have common features such as significant changes in the expression of genes involved in the acquired immune response to infection (e.g., antigen uptake and presentation, pattern recognition receptors). Alterations in the expression of infection-related genes with our scald-burn model is interesting, as infection is rarely observed as early as within 24 h after burn injury (especially with animals grown in a controlled, infection-free environment, as was done in this
study). Our observations can be explained by several published reports that suggest the possibility of bacterial flora leaking from the gut into the blood stream after burn injury and triggering an inflammatory response (14, 15, 43).

Similarities and differences in the expression of metabolism-related genes are also observed between our scald-burn model and the LPS infection model. Genes involved in FA biosynthesis and cholesterol biosynthesis are downregulated in both studies; however, genes involved in FA oxidation and bile acid synthesis are upregulated in our study, whereas these processes are downregulated in the LPS model (47). The increase in FA oxidation in our study could be a direct consequence of higher energy demand that is possibly required for wound healing after burn injury, compared with the LPS model.

In summary, the framework of coordinated changes in gene expression and metabolites described in this study provides a possible link between changes in the metabolic and inflammatory responses observed during the acute response to burn injury (Fig. 9), with the oxidation of FA primarily occurring to meet the energy demands arising from the acute inflammatory response. Based on these results, we hypothesize that the dynamics of hepatic energy metabolism vary depending upon the nature and intensity of the driving force (i.e., the inflammatory response); accordingly, a severe inflammatory model such as burn injury could result in the rapid depletion of FA-based energy reserves and trigger the early onset of muscle wasting for supplying energy substrates to the liver. Currently, work is in progress to rigorously define these time scales in terms of the gene expression as well as metabolite profiles in the liver following burn injury.

ACKNOWLEDGMENTS

We thank Charles Roth for comments. Use of facilities at the Special Shared Facility for Functional Genomics and Proteomics at the Shriners Burns Hospital is also acknowledged.

GRANTS

This work was supported in part by National Institutes of Health Grant RO1-GM-65474 (to M. L. Yarmush) and by Whitaker Foundation Grant RG01-01-117 (to A. Jayaraman).

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