Stimulation of Rat Hepatic Amino Acid Transport by Burn Injury

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Burn injury accelerates hepatic amino acid metabolism, but the role of transmembrane substrate delivery in this response has not been investigated. We therefore studied the effects of cutaneous scald injury on the Na^+-dependent transport of glutamine and alanine in isolated rat liver plasma membrane vesicles. Scald injury resulted in liver damage and a 1.4- to 2.3-fold and 1.5- to 2.8-fold stimulation of hepatic transport rates for glutamine and alanine, respectively, proportional to the total burned surface area (TBSA) after 24 hours. Enhanced uptake of glutamine and alanine was attributable to increases in the maximum velocity (V_{max}) of system N and system A activities, respectively. Hepatic amino acid transport activity remained elevated in vesicles from burned animals after 72 hours, but the degree of stimulation (1.3- to 1.7-fold for glutamine and 1.3- to 1.6-fold for alanine) was less than that observed 24 hours after thermal injury. Liver function tests returned to control values after 72 hours as well, indicating rectification of hepatic damage. In contrast to the induction of hepatic system A and system N activity in catabolic states such as cancer and endotoxemia, further studies showed that tumor necrosis factor (TNF) failed to play a significant role in burn-stimulated amino acid transport rates. When combined with plasma liver enzyme profiles, early transient hepatic amino acid transporter stimulation may support amino acid–dependent pathways involved in the repair of burn-dependent hepatic damage.

AS THE PRIMARY CENTER of glucose and nitrogen homeostasis in the body, the liver has been shown to display marked changes in metabolism after burn injury. For example, de novo biosynthesis of purine and pyrimidine nucleotides in the liver of rats following burn injury was increased after 24 hours[1] and the amount of DNA and RNA in rat liver nuclei was elevated up to 48 hours.[2] Concurrently, the level of mRNA for major acute-phase α_1-protein increased 20-fold, for fibrinogen eightfold, and for α_1-acid glycoprotein about ninefold.[3] A central feature of this acute-phase response to burn injury is a mobilization of amino acids from the periphery[4] and an increased uptake of amino acids by the liver to support acute-phase protein production, ureagenesis, and gluconeogenesis.[5] The liver also suffers acute cell damage following burn injury, as characterized by elevated plasma levels of transaminases and an acute-phase response characterized by diminished production of albumin.[6] Histological examination of the liver after burn injury revealed a proliferation of phagocytic Kupffer cells, necrotic hepatocytes,[7] and foci of cells with anomalous and degenerated mitochondria.[8] Whether hepatic damage precedes, occurs concurrently with, or is elicited as a result of the burn-induced acute-phase response remains to be fully elucidated.

Although burn-dependent induction of amino acid extraction by the liver has been reported,[9] the contribution of the major amino acid transporters that reside in the hepatocyte plasma membrane to this response has not been studied. Several distinct amino acid transport systems have been kinetically characterized in the liver, including the Na^+-dependent systems A, N, and ASC.[10] It has become increasingly apparent that the activities of the membrane proteins catalyzing the selective transport of amino acids play a major mechanistic role in regulation of hepatic physiology,[11] especially when intracellular metabolism is accelerated.[12,13] Here, we investigated the effects of scald injury on the Na^+-dependent transport of glutamine and alanine, two amino acids whose transmembrane delivery may be conditionally rate-limiting for metabolism.[13,14] Also, given the importance of glutamine (system N) and alanine (systems A and ASC) in hepatic gluconeogenesis and ureagenesis—two processes accelerated by burn injury[5]—the studies presented here were undertaken to examine the impact of burn injury on the transport of these amino acids across the liver cell plasma membrane.

Similar to other catabolic states, the systemic signaling pathways that ultimately elicit changes in hepatic physiology and metabolism after thermal injury are likely to be complex, integrated, and multifactorial in nature.[15] As an initial investigation into the potential roles for inflammatory cytokines in burn-accelerated hepatic amino acid transport rates, two candidates were studied: tumor necrosis factor alpha (TNFα), previously shown to participate in endotoxin- and tumor-induced hepatic amino acid transport activity,[16,17] and interleukin-1β (IL-1β), shown to enhance hepatic alanine transport and gluconeogenesis in vivo in previous studies.[18] Both of these cytokines have also been reported to be elevated in the plasma of burn patients early after injury,[19] and are thought to initiate and perpetuate the postburn hypermetabolic state.[15]

MATERIALS AND METHODS

Burn Model

Adult male Sprague-Dawley rats (175 to 250 g; Charles River Laboratories, Wilmington, MA) were used for these studies. For one set of studies, slightly older and larger rats (316 ± 4 g) were used that had been maintained in the animal facility slightly longer than the others. All experiments were approved by the Committee on Research, Subcommittee on Research Animal Care, Massachusetts General Hospital, and conform to National Institutes of Health guidelines. Animals were

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housed in the animal care facility under standard conditions (ie, 12-hour light-dark cycle and access to standard rat chow and water ad libitum) and were allowed at least 2 days to acclimate to the surroundings. Prior to studies, rats were randomly separated into different groups. Animals were weighed and anesthetized with ketamine (75 mg/kg, Ketaset; Fort Dodge Laboratories, Fort Dodge, IA), acepromazine maleate (10 mg/kg, PromAce; Aveco, Fort Dodge, IA), and xylazine (1.3 mg/kg, Rompun; Mabay, Shawnee, KA). The animals were shaved, and full-thickness scald burns were administered by immersion of the animal's back for 10 seconds (approximately 20% ± 1% total body surface area [TBSA]) or, for a large burn, of the abdomen as well for 5 seconds (approximately 12% TBSA burn; 31% ± 3% total) in water at 100°C.[20] The full-thickness nature of these burns render the injuries anesthetic.[21] The major and minor axes of the resulting elliptical thermally injured areas were measured and used to calculate the size of the injury in centimeters squared (0.7854 × major diameter × minor diameter). Control (sham) burns were administered by immersion of the animals in water at room temperature. Burn size in percent TBSA was calculated via the empirical formula for total animal surface area[22]: 11 × weight (grams)$^{0.631}$. After the scald, the animals were fluid-resuscitated immediately and again after 5 hours by intraperitoneal injection with 12 mL warm (37°C) lactated Ringer's solution. Hepatic plasma membrane vesicles (HPMVs) were prepared 24 and 72 hours after burn injury.

Preparation of HPMVs

HPMVs were prepared by Percoll density-gradient centrifugation with minor modifications as described in detail previously.[23] Briefly, at 24 and/or 72 hours, the livers were perfused with ice-cold phosphate-buffered saline (150 mmol/L NaCl and 10 mmol/L Na_2HPO_4, pH 7.4), excised, minced, and homogenized in 20 mL SEB (250 mmol/L sucrose, 1 mmol/L EGTA, and 10 mmol/L HEPES, pH 7.5) with a Dounce homogenizer by 10 strokes with a loose-fitting pestle followed by four strokes with a tighter-fitting pestle. The homogenate was brought to 100 mL with SEB and centrifuged at 150 × *g* for 2 minutes to remove gross particulate matter, and the resulting supernatant was centrifuged at 1,500 × *g* for 10 minutes. The crude membrane pellet was filtered through 8-ply gauze, added to 8.4 mL Percoll (Sigma, St Louis, MO), and brought to a final volume of 60 mL with SEB (11.9% vol/vol final [Percoll]). The suspension was thoroughly mixed and centrifuged at 34,000 × *g* for 30 minutes. Plasma membrane bands were harvested, diluted 1:6 (vol/vol) with SMB (250 mmol/L sucrose, 1 mmol/L $MgCl_2$, and 10 mmol/L HEPES, pH 7.5), and washed free of Percoll via a second centrifugation. Membrane vesicle pellets were resuspended in SMB, and aliquots were stored at −80°C until studied. Vesicle purity was evaluated biochemically by the relative enrichment or impoverishment of the measured specific activity of the plasma membrane marker enzymes 5′ nucleotidase,[24] gamma glutamyl transferase (GGT),[25] and Na^+/K^+-ATPase,[26] as well as the microsomal enzyme marker glucose-6-phosphatase.[27] Inorganic phosphate was measured according to the method of Fiske and Subbarow.[28]

Amino Acid Transport Assay

Initial-rate amino acid transport by HPMVs was evaluated by a rapid mixing/filtration technique described previously.[16,17,23,29] Transport assays were undertaken in the absence or presence of Na^+. Uptake was initiated by mixing 20 μL vesicles with 20 μL Na^+- or K^+-uptake buffer containing amino acid tracer in 12 × 75-mm polystyrene tubes using an electronic timer/vortexer apparatus. Final concentrations in the reaction mixture were 50 mmol/L NaCl or KCl, 1 mmol/L $MgCl_2$, 10 mmol/L HEPES (pH 7.5), and varying concentrations of test amino acid depending on the experiment. Amino acid uptake was terminated by addition of 1 mL ice-cold wash buffer (119 mmol/L NaCl, 25 mmol/L Na_2HPO_4 (pH 7.5), 5.9 mmol/L KCl, 0.5 mmol/L $CaCl_2 \cdot 2H_2O$, and 1.2 mmol/L $MgSO_4 \cdot 7\ H_2O$) followed by immediate low-pressure vacuum filtration of the mixture over a 0.45-μm nitrocellulose filter to separate intravesicular from extravesicular radiolabeled amino acid. The filter was rapidly washed twice with 3 mL wash buffer and dissolved in 1 mL Microscint 20 (Packard Instruments, Downers Grove, IL) for determination of trapped radioactivity by scintillation spectrophotometry (Packard TopCount; Packard Instruments). Classic "Na^+-overshoot" profiles[30] were obtained for the time courses at both 37°C and 22.5°C, but the overshoot occurred at 10 and 30 seconds for both temperatures, respectively. Therefore, to ensure measurement of initial-rate values at reasonable time points, all subsequent transport assays were performed at 22.5°C, and with the exception of time courses, transport assays were terminated after 10 seconds. L-[G-^{3}H]glutamine and L-[2,3-^{3}H]alanine were purchased from Amersham (Arlington Heights, IL).

The Na^+-dependent component of amino acid transport was calculated by subtracting uptake velocities in the presence of K^+ (Na^+-independent uptake, triplicate determinations) from those in the presence of Na^+ (total uptake, triplicate determinations). System N activity was operationally defined as the Na^+-dependent transport of 50 μmol/L L-glutamine uninhibited by the presence of 10 mmol/L α-(methylamino)isobutyric acid (MeAIB), a selective and nonmetabolizable substrate of the system A carrier.[10] System A and ASC activities were functionally defined as the portion of Na^+-dependent transport of 50 μmol/L L-alanine inhibited or uninhibited by the presence of 10 mmol/L MeAIB, respectively.

Transport kinetic characteristics were determined by varying the final concentration of unlabeled amino acid from 0.01 mmol/L to 40 mmol/L, and were also performed at the 10-second time point under initial-rate conditions. Osmotic adjustments for varying concentrations of test amino acids were made with sucrose where appropriate. Kinetic data for amino acids that displayed biphasic (curvilinear) plots—indicative of mediation by two distinct carriers—were analyzed by nonlinear regression analysis with the equation for transporters that display Michaelis-Menten kinetics,

$$v=((V_{max1} \cdot [S])/(K_{m1} + [S])) + ((V_{max2} \cdot [S])/(K_{m2} + [S])),$$

where V_{max} 1 and 2 represent the maximum velocities (capacities) and K_m 1 and 2 represent the transporter affinities for the amino acid of both distinct carriers, respectively, and [S] represents the concentration (millimoles per liter) of the amino acid under study.

In all transport experiments, blank values (no vesicles present) were determined and subtracted from the corresponding amino acid uptake values. Membrane protein was determined by a bicinchoninic acid assay (BCA protein assay reagent; Pierce Chemical, Rockford, IL). Transport values were normalized to vesicular protein content, and the resulting rates are expressed as picomoles of amino acid per milligram protein per 10 seconds. All transport assays were performed in triplicate.

Studies With Antibodies Against TNFα

In a second series of experiments, control and burned rats were treated with a rabbit polyclonal antibody against murine TNFα that cross-reacts with rat TNFα. The antibody was a gift from Dr H. Richard Alexander (National Cancer Institute, Surgery Branch, Bethesda, MD). The antibody has a half-life of about 3 days in vivo. Rats received the anti-TNFα antibody intraperitoneally (2 mg per rat) 2 hours prior to large burn injury. A control group of sham-burned and burned animals received non-immune rabbit immunoglobulin G ([IgG] Sigma Chemical, St Louis, MO). In all groups (n = 4 per group), HPMVs were prepared 24 and 72 hours after burn injury.

Determination of Serum Cytokine Levels

Serum levels of rat TNFα and IL-1β were determined by enzyme-linked immunosorbent assay kits from Biosource International (Camarillo, CA).

Liver Function Tests

For determination of serum transaminase activities (glutamate-oxaloacetate transaminase [GOT] and glutamate-pyruvate transaminase [GPT]) and albumin levels, reagent kits from Sigma Chemical were used.

Statistical Analysis

Data were analyzed by the Mann-Whitney test, and transport velocities and kinetic parameters were determined from uptake data by linear and nonlinear regression analysis using computer software (SPSS, SPSS, Chicago, IL; Harvard Chart XL, Software Publishing, Santa Clara, CA; and DataDesk, Data Description, Ithaca, NY). The level of significance was set at P less than .050. All data are expressed as the mean ± SEM and are from at least three separate determinations per animal.

RESULTS

Assessment of HPMV Integrity

The vesicular nature and functional reliability of our vesicle preparations in terms of the capacity for time- and concentration-dependent carrier-mediated accumulation of amino acids, enrichment in plasma membrane markers, and electron microscopic appearance have been documented previously.[16,17,23,29] In this report, the specific activity of selected marker enzymes in vesicle preparations from control and burned animals was measured to evaluate vesicle enrichment in plasma membrane. Vesicle preparations were enriched ninefold to 12-fold in the specific activity of the plasma membrane enzyme marker 5′-nucleotidase compared with crude liver homogenates, and no differences in the degree of enrichment were observed in HPMVs from control or burned animals. Furthermore, vesicles isolated according to this protocol displayed 11-fold and 15-fold enrichment in the specific activities of plasma membrane markers GGT and Na^+/K^+-ATPase, respectively. Concomitant with the enrichment in plasma membrane, vesicle preparations from both control and burned animals exhibited no enrichment in the specific activity of the microsomal membrane enzyme marker glucose-6-phosphatase.

Effect of Burn Injury on Amino Acid Transport in HPMVs

Small burn injury (20% ± 1% TBSA) resulted in an increase in total hepatic Na^+-dependent glutamine transport activity of 35% after 24 hours ($P < .010$; Fig 1) and 29% after 72 hours ($P < .010$; Fig 2) compared with sham-burn injury. In large burns, the increase was even more marked, 134% after 24 hours ($P < .010$; Fig 1) and 67% after 72 hours ($P < .010$; Fig 2). Thus, small burns elicit a significant increase in hepatic glutamine uptake after 24 hours, an effect that persists at 72 hours postburn, whereas large burns elicit a more dramatic initial increase in hepatic glutamine transport activity, but evidence of a decline toward small burn–induced values is observed by day 3. After adding excess MeAIB to the uptake mixture to block any System A–mediated glutamine transport, assays showed that in vesicles from animals subjected to small burns there was an increase in System N activity of 44% after 24 hours ($P < .010$; Fig 1) and 27% after 72 hours ($P < .010$; Fig 2), suggesting that nearly all of the small burn–induced glutamine uptake is attributable to augmented System N activity. In vesicles from animals subjected to large burns, MeAIB-insensitive glutamine transport was increased by 75% after 24 hours ($P < .010$; Fig 1) and by 42% after 72 hours ($P < .010$; Fig 2) compared with vesicles from sham-burned animals. These results indicate that in rats subjected to large thermal injury, System N induction contributes largely to the observed increase in glutamine uptake, but some System A–mediated glutamine uptake is also evident early.

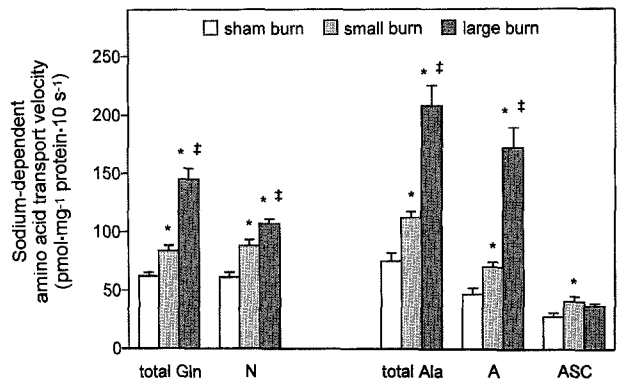

Fig 1. Hepatic amino acid transport activity 24 hours after scald injury. Plasma membrane vesicles were isolated from animals 24 hours after subjection to sham, small (20% ± 1% TBSA), or large (31% ± 3% TBSA) scald injury, and amino acid transport was measured by the rapid mixing/filtration method. Substrate concentrations for both L-glutamine and L-alanine were 50 μmol/L. Total Na^+-dependent initial-rate transport velocities for both amino acids are presented, and the contribution of individual transporters to these velocities is illustrated. Data are the mean ± SEM of triplicate determinations from 4 individual animals (n = 12). *$P < .010$ *v* sham-burn, ‡$P < .010$ *v* small burn.

For alanine, a major gluconeogenic substrate shuttled to the liver,[31] there was an even more marked increase in Na^+-dependent transport activity following burn injury. After a small burn injury, the transport rate increased 49% after 24 hours ($P < .010$; Fig 1) and 28% after 72 hours ($P < .010$; Fig 2). In vesicles from animals subjected to large thermal injury, the

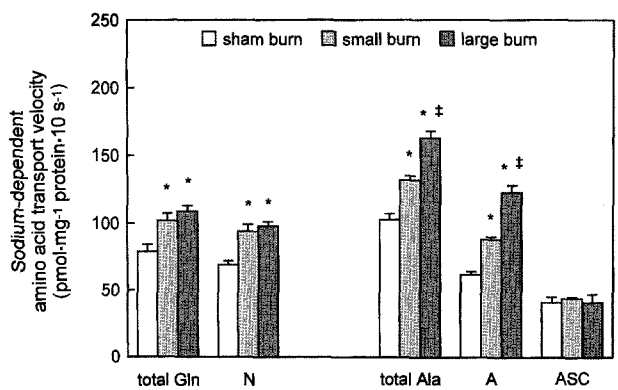

Fig 2. Hepatic amino acid transport activity 72 hours after scald injury. Plasma membrane vesicles were isolated from animals 72 hours after subjection to sham or scald injury. Initial-rate transport velocities for L-glutamine and L-alanine were measured as described in Fig 1. Data are the mean ± SEM of triplicate determinations from 4 individual animals (n = 12). *$P < .010$ *v* sham-burn, ‡$P < .010$ *v* small burn.

observed increase was more pronounced, with 180% induction ($P < .010$; Fig 1) after 24 hours and 58% after 72 hours ($P < .010$; Fig 2). Similar to glutamine, increased hepatic alanine transport activity in both small- and large-burn animals displayed evidence of decline by day 3.

The activity of System ASC (MeAIB-insensitive Na^+-dependent alanine transport) changed only after 24 hours. It increased by 46% in small burns ($P < .010$) and by 32% in large burns (NS). There was no significant difference in System ASC activity in either group 72 hours after burn.

System A activity (MeAIB-inhibitable Na^+-dependent alanine transport) in vesicles from animals subjected to small burn injury displayed an increase of 48% after 24 hours ($P < .010$; Fig 1) and 42% after 72 hours ($P < .010$; Fig 2). In large-burn animals, System A activity increased by 260% after 24 hours ($P < .010$; Fig 1) and by 98% after 72 hours ($P < .010$; Fig 2). The increase in transport activity for alanine following burn injury was therefore almost exclusively attributable to System A induction.

Kinetic Basis for Transporter Activation

The increase in both Na^+-dependent glutamine and alanine transport was kinetically analyzed at 24 hours after large scald injury and determined to be attributable to an increase in the capacity (V_{max}) relative to changes in substrate affinity of the respective carriers (K_m values). The Eadie-Hofstee linear transformation of the data is presented in Fig 3, and shows that glutamine transport (Fig 3A) displays linear kinetics (indicative of a single carrier), whereas alanine (Fig 3B) displays curvilinear kinetics (indicative of two distinct carriers). Such a biphasic plot for hepatic alanine transport has been previously described by our group[23] and others.[32,33] Regression analysis showed that the V_{max} of System N increased from 642 ± 125 pmol · mg^{-1} protein · 10 s^{-1} in vesicles from sham-burned animals to 1,044 ± 178 pmol · mg^{-1} protein · 10 s^{-1} in vesicles from burned animals ($P = .059$), while the K_m values changed only slightly between the two groups (0.405 ± 0.088 *v* 0.306 ± 0.064 mmol/L, P = NS, in vesicles from sham-burned and scald-burned animals, respectively). Similarly, nonlinear regression analysis showed that the observed burn-induced stimulation of alanine uptake was due to an increase in the V_{max} of the high-affinity system (Fig 3B). Values for V_{max} of the high-affinity system were 193 ± 25 and 499 ± 41 pmol · mg^{-1} protein · 10 s^{-1} ($P < .001$) in vesicles from sham and burned animals, respectively, while those for the low-affinity carrier changed very little (4,830 ± 1,450 *v* 5,820 ± 980 pmol · mg^{-1} protein · 10 s^{-1}, P = NS, in vesicles from sham and burned animals, respectively). The affinities of each of the carriers for alanine were relatively unaffected by burn injury, with K_m values for the low-affinity system of 12.61 ± 2.79 versus 14.80 ± 0.45 mmol/L (P = NS) and for the high-affinity system of 0.061 ± 0.027 versus 0.054 ± 0.013 mmol/L (P = NS) in vesicles from sham-burned and scald-burned rats, respectively. When combined with the data presented in Fig 1, these results suggest that the induction of a high-affinity transport component—probably System A—underlies the observed increase in hepatic alanine uptake in response to burn injury.

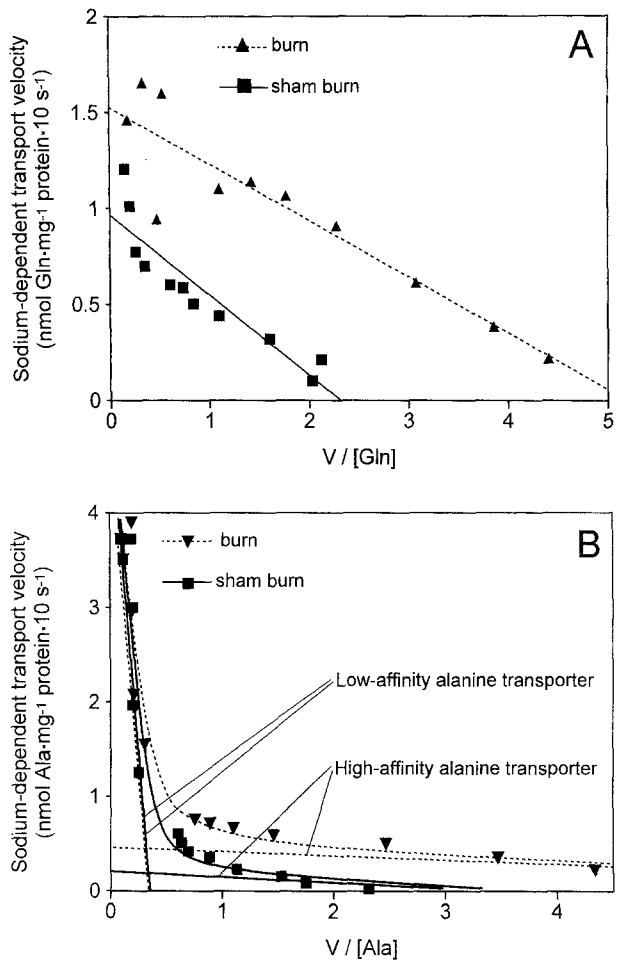

Fig 3. Kinetic analysis of burn-stimulated glutamine and alanine transport activity in HPMVs from rats 24 hours after subjection to large (31% ± 3% TBSA) scald injury. Initial-rate Na^+-dependent transport velocities were determined at increasing L-glutamine (0.01 to 8 mmol/L, A) and L-alanine (0.01 to 30 mmol/L, B) concentrations. Results are presented as the Eadie-Hofstee linear transformation of the resulting kinetic data. (A) Linear regression analysis for glutamine uptake showed an increase of the Vmax (642 ± 125 *v* 1,044 ± 178 pmol · mg^{-1} protein · 10 s^{-1} in vesicles from control and burned animals, respectively) relative to a slight change in substrate affinity (0.405 ± 0.088 mmol/L (sham) *v* 0.306 ± 0.064 mmol/L (burn), P = NS). (B) Nonlinear regression analysis of the biphasic alanine kinetic plot showed that an increase in the V_{max} of the high-affinity component underlies the observed burn-induced increase (193 ± 25 and 499 ± 41 pmol · mg^{-1} protein · 10 s^{-1}, $P < .001$, for vesicles from sham and burned animals, respectively). A slight but insignificant change in the V_{max} for the low-affinity component was observed (4,830 ± 1,450 (sham) *v* 5,820 ± 980 (burn) pmol · mg^{-1} protein · 10 s^{-1}, P = NS), as well as the alanine affinities of both carriers (12.61 ± 2.79 *v* 14.80 ± 0.45 mmol/L, P = NS, for the low-affinity system and 0.061 ± 0.027 *v* 0.054 ± 0.013 mmol/L, P = NS, for the high-affinity system in vesicles from sham and scalded rats, respectively). The curves for total alanine uptake and the contribution of each system to the respective profiles are illustrated. Data are the mean of triplicate determinations for each substrate concentration, and error bars (typically 5% to 20% of the mean) have been eliminated for clarity.

Effect of Anti-TNF Antibodies on Burn-Induced Amino Acid Transport

TNF has previously been shown to play a role in the induction of endotoxin- and tumor-induced increases in hepatic amino acid transport activities.[16,17] To investigate a potential role for TNFα in the induction of amino acid transport after burn injury, rats were pretreated with antibodies against TNF prior to large scald injury.

Burn injury–induced glutamine transport activity was attenuated by 18% in the group of animals pretreated with anti-TNF compared with animals pretreated with control IgG after 24 hours ($P < .010$; Fig 4). The effect of anti-TNF pretreatment was no longer evident 72 hours after burn injury (data not shown). To investigate whether the effect of antibodies against TNF was due to an attenuation of System N– or System A–mediated glutamine uptake, the transport assays were performed in the presence or absence of excess (10 mmol/L) MeAIB. The results in Fig 4 indicate that the anti-TNF–attenuated portion of glutamine uptake was MeAIB-insensitive and therefore attributable to System N ($P < .010$).

The transport of alanine in vesicles from anti-TNF–pretreated burned animals was 13% lower than in vesicles from IgG-pretreated burned animals after 24 hours. However, this difference failed to reach statistical significance ($P = .089$). Similar to burn-induced glutamine uptake, there was no difference in alanine uptake observed between the anti-TNF– or IgG-treated groups after 72 hours.

Serum Cytokine Levels in Burned Rats

Because the anti-TNF antibodies used in this study were previously shown to attenuate both tumor- and endotoxin-induced hepatic amino acid transport[16,17] and exerted only minor effects on burn-induced activities, we sought to determine the basis for these disparities. Serum was therefore obtained from rats injected with 10 mg/kg bacterial lipopolysaccharide (LPS), rats bearing a methylcholanthrene-induced fibrosarcoma ([TBR], 15% of total body weight), rats subjected to large burn injury, and sham-burned rats after 24 hours. The results showed that TNFα levels in plasma from endotoxin-treated and tumor-bearing rats exceed those in burned rats by severalfold (540, 290, and 20 pg/mL in TBR, LPS, and burned animals, respectively). Values for the control-treated groups in each case ranged from 10 to 18 pg/mL. These results could therefore explain the relative ineffectiveness of the anti-TNF antibodies in burned animals compared with the significant attenuation of induced transport values in tumor-bearing and endotoxemic animals.

Serum IL-1β levels were elevated in LPS-treated animals (100 pg/mL) compared with normal or sham-burned rats (undetectable). Furthermore, immunoreactive IL-1β was undetectable in the serum of tumor-bearing rats and in the serum of animals subjected to small or large burns after 24 hours. These results collectively indicate that the etiology of the catabolic insult profoundly influences the subsequent systemic elaboration of inflammatory cytokines.

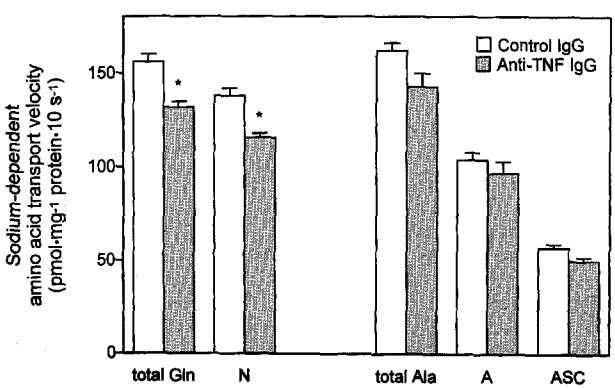

Fig 4. Effect of pretreatment with TNF-neutralizing antibody on burn-induced hepatic amino acid transport activity. Animals received 2 mg rabbit anti-murine TNFα IgG or control rabbit IgG intraperitoneally 2 hours before 31% ± 3% TBSA burn injury, and HPMVs were prepared 24 hours thereafter. Initial-rate (50 μmol/L) Na^+-dependent transport velocities for both L-glutamine and L-alanine were determined in HPMVs from each group. Data are the mean ± SEM of triplicate determinations from 4 separate animals in each group (n = 12). *$P < .010$ *v* control IgG.

Effect of Animal Size on Burn-Induced Hepatic Amino Acid Transport

In the course of these studies, larger animals (316 ± 4 g) were subjected to a small burn injury similar to that of the smaller animals used earlier in the studies (220 ± 31 g). Despite nearly identical TBSA injuries (18.0% ± 1.5% *v* 19.6% ± 1.5% in large *v* small animals, respectively, P = NS), glutamine transport in HPMVs from the larger animals failed to be accelerated compared with HPMVs from the sham-burned controls (101.7 ± 14.3 *v* 101.3 ± 13.7 pmol · mg^{-1} protein · 10 s^{-1} in burn *v* sham, respectively) after 24 hours. However, alanine transport rates were still induced by burn injury in these larger animals (140.4 ± 14.2 *v* 114.8 ± 21.0 pmol · mg^{-1} protein · 10 s^{-1}, $P < .010$, burn *v* sham, HPMVs, respectively), but to a lesser extent than in the smaller animals (Fig 1; ie, 22% *v* 49% increase, respectively). Figure 5 demonstrates the relationship between the TBSA to body weight ratio, hepatic damage, and the effect on subsequent hepatic glutamine or alanine transport rates after 24 hours.

Liver Function Tests

Burn-induced changes in hepatic amino acid transport activity occur within the context of liver damage (Figs 5 and 6). To measure the insult to the liver in this burn model, serum levels of (released) transaminase enzymes (GOT and GPT) were measured at 8, 24, and 72 hours after scald injury. An additional parameter of hepatic function is serum albumin levels, which were determined in all rats in this study. After 8 hours, there was a 2.3-fold and 3.6-fold increase in serum GOT and GPT ($P < .010$ and $P < .050$), respectively, in response to large burns, with no significant diminution in serum albumin levels. After 24 hours, in small burns the serum concentration of GOT was elevated twofold ($P < .010$) and in large burns threefold ($P < .010$) compared with sham-burned animals. The concentration of GPT increased 2.8-fold ($P < .010$) in small burns and fourfold ($P < .010$) in large burns after 24 hours (Fig 6). Likewise, the concentration of albumin in the blood decreased

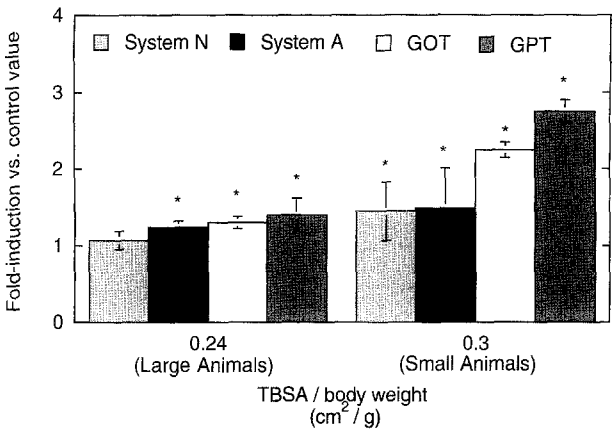

Fig 5. Effect of animal weight on small burn–induced liver damage and amino acid transport rates. Data are the ratios (±SD) of burn to control means for each parameter depicted and represent the average fold-induction produced by burn injury. These values are represented as a function of the TBSA to animal weight ratio, which was 0.296 ± 0.013 in smaller rats *v* 0.236 ± 0.022 in larger rats ($P < .010$). The burn size was 65 ± 8 cm² in the small rat (220 ± 31 g, n = 4) group and 74 ± 6 cm² in the large rat (316 ± 4 g, n = 3) group. *$P < .010$ *v* sham-burn.

as a result of burn injury, perhaps indicative of the acute-phase response; in small burns it decreased by 24% ($P < .010$) and in large burns by 21% ($P < .010$), indicating that there is no apparent direct correlation between burn size and serum albumin diminution. By 72 hours, serum transaminase activities normalized toward control values while albumin levels remained slightly depressed, indicating that the liver damage observed acutely was rectified while the acute-phase response continued.[6]

To test whether greater hepatic damage was observed in large burns simply because the abdomen was involved rather than an increase in the percent TBSA per se, GOT and GPT levels were measured 24 hours after small thermal injuries of 9.1% ± 1.8% TBSA on either the abdomen or the back. GOT activity

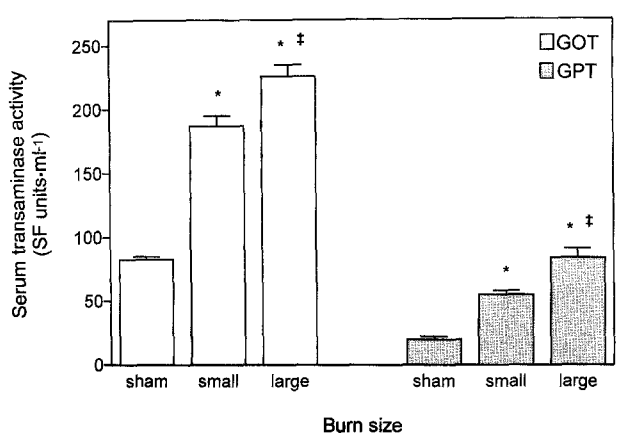

Fig 6. Effect of burn size on liver damage after 24 hours. Animals were subjected to sham, small (20% ± 1% TBSA), or large (31% ± 3% TBSA) scald injury, and serum was collected 24 hours later and analyzed for released hepatic transaminase activity. SF units are "Sigma-Frankel" units, per the manufacturer's protocol. *$P < .010$ *v* sham-burn, ‡$P < .010$ *v* small burn.

increased from 59 ± 5.1 in shams to 124 ± 11 "Sigma-Frankel" units (SFU)/mL ($P < .010$) in dorsally injured animals and to 148 ± 16 SFU/mL in ventrally injured animals ($P < .010$). Likewise, GPT values increased from 32 ± 3 in shams to 61 ± 13 ($P < .010$) and 56 ± 12 ($P < .010$) SFU/mL in dorsally and ventrally injured animals, respectively. Thus, ventral and dorsal injuries of the same percent TBSA induce equivalent degrees of liver damage.

Similar to its effects on stimulation of hepatic amino acid transport activity, the size of the animal additionally influences the extent of liver damage induced by an otherwise identical burn. Despite similar burn surface areas (18.0% ± 1.5% *v* 19.6% ± 1.5% in large *v* small animals, respectively), serum GOT and GPT levels were increased only 1.3 ± 0.1- and 1.4 ± 0.2-fold, respectively, in animals that weighed 316 ± 4 g versus 2.0 ± 0.1- and 3.0 ± 0.2-fold in animals that weighed 220 ± 31 g compared with sham-burned control serum values (Fig 5). These results demonstrate that the size of the animal impacts the subsequent extent of liver damage, as well as the degree of transporter stimulation, induced by thermal injury.

DISCUSSION

Previous studies have shown that hepatic amino acid transport activities are enhanced during catabolic states such as cancer[17] and endotoxemia,[16] observations that underscore the importance of the liver in the maintenance of glucose and nitrogen homeostasis in the body during times of metabolic stress. It is well established that burn injury—another catabolic state—leads to hypermetabolism and negative nitrogen balance, the extent of which depends on the size of the burn.[9] Hepatic metabolism increases, as does the level of mRNA for major acute-phase proteins.[3] As part of the response to burn injury, amino acids are released from peripheral organs and taken up by the liver.[4] Two of the amino acids most profoundly influenced during catabolic states are alanine and glutamine,[4] which is not surprising given the role of each in accelerated gluconeogenesis.[5,31,34] Since the possibility of a regulatory role for plasma membrane transport systems in hepatic amino acid metabolism has been raised,[12-14] we undertook the studies presented here to define for the first time the effects of burn injury on hepatic transport systems for the two primary gluconeogenic amino acid substrates. To this end, isolated HPMVs were used since they offer the unique advantage of discriminating membrane transport activities per se, independent of other confounding influences (eg, alterations in blood flow, intracellular metabolism, intracellular amino acid *trans*-effects, etc.).

However, there exists a possibility that the subcellular fractionation of an organ in the context of tissue damage may lead to artifactual results due to physicochemical changes in membrane properties and their impact on purification. However, several pieces of evidence suggest that this is not a concern. As an extension of the studies presented here, amino acid transport was examined in isolated hepatocytes from control and burned rats, and similar inductions in alanine and glutamine uptake were observed in these intact cell preparations (Lohmann, Bode, and Souba, unpublished data, March 1997). As a result, it is unlikely that the accelerated rates of uptake observed in plasma membrane vesicles from burned animals are a damage-induced artifact of the HPMV isolation procedure.

Also, burn injury is not the only catabolic state associated with some degree of hepatic damage; for example, liver damage in response to endotoxemia is well established (ie, a Medline search revealed 83 references on this topic). Several studies on the endotoxin model of sepsis and its impact on hepatic amino acid uptake in plasma membrane vesicles have been published,[16,23,35] and subsequent studies on endotoxemia and hepatic amino acid transport in isolated hepatocytes corroborated the observations in HPMVs.[36] Additionally, alterations in membrane transport activity induced in intact hepatocytes and liver are retained during preparation of vesicles as previously shown by our laboratory and others,[16,17,37] so HPMVs essentially provide a "snapshot" of changes in membrane transporters elicited in vivo.

Kinetic analysis revealed that burn injury induces the transport of both alanine and glutamine, primarily via increases in the activities of System A and System N, respectively. Because the genes and corresponding proteins responsible for systems A and N have yet to be isolated, we presently must rely on kinetic analysis to characterize increases in the activity of each. Based on the kinetic data, the enhanced activity is attributable to an increase in the maximum velocity (V_{max}) of each carrier, an index of the capacity to transport a given substrate, which is usually attributable (but not restricted) to the presence of additional transporters in the plasma membrane. In the case of alanine, the data collectively indicate that augmentation of the high-affinity MeAIB-inhibitable component—probably System A—underlies the accelerated transport of this amino acid after burn injury, although radiolabeled MeAIB was not used in these studies to directly test this conclusion. Stimulation of this carrier versus the low-affinity component makes sense physiologically, as the affinity for alanine of 50 to 60 μmol/L (albeit in the absence of intravesicular amino acids) more closely reflects plasma levels of this amino acid (200 to 300 μmol/L). Induction of similar high-affinity System A components in hepatocytes has been previously reported to be elicited in response to partial hepatectomy[32] and chronic starvation,[33] raising the possibility that this particular carrier may be generally expressed in response to metabolic stress and catabolism. Likewise, induction of System N V_{max} allows this transporter to more efficiently take up glutamine, especially when its intracellular utilization is accelerated—a condition under which transport via this carrier has been shown to be rate-limiting for metabolism.[13] It is likely that augmentation of System N transport capacity—in the absence of increased plasma glutamine levels—allows the liver to meet the burn-induced metabolic demand for its substrate.

While the data presented in this study indicate that transporters for glutamine and alanine are enhanced, the signals that elicit these changes remain poorly defined. However, one concept that has emerged from these studies is that the mechanisms responsible for activation of hepatic amino acid transport in catabolic states such as cancer[17] and endotoxemia[16] are distinct from those responsible for burn-dependent stimulation. Based on the data, TNFα does not seem to play a significant role in the early hepatic response to burn injury. Although this cytokine has been reported to be elevated in the plasma of burn patients,[19] the differential role of TNF in the induction of hepatic amino acid transport in specific catabolic states may be explained by the relative levels of this cytokine in the serum from burned (20 pg/mL), tumor-bearing (540 pg/mL), and endotoxemic (290 pg/mL) rats. This proinflammatory cytokine has previously been shown to increase hepatic amino acid transport when directly injected into rats,[29] but, consistent with our results, serum TNF levels have been shown to be much higher in infected burned animals than in uninfected burned animals.[38] There is also evidence that a distinct form of TNF may be elaborated in burned animals.[39] Likewise, serum levels of another inflammatory cytokine, IL-1β, have been reported to be elevated in patients after burn injury[19] and to accelerate hepatic alanine transport and metabolism in vivo.[18] However, serum levels of IL-1β were elevated in LPS-treated animals but not in tumor-bearing or burned animals after 24 hours. This finding does not rule out the possibility that there are transient increases in this cytokine immediately after burn injury or that it plays a role later in the healing process. Further studies with an IL-1 receptor antagonist are planned to more definitively test the possible involvement of this cytokine. Although the direct mediators of transporter stimulation remain unclear, there is preliminary evidence that the putative burn-dependent signaling factor(s) may be plasma-borne: when slices of fresh liver were incubated in the presence and absence of plasma from burned rats, accumulation of the synthetic nonmetabolizable System A substrate α-aminoisobutyric acid (AIB) was enhanced in the presence of burn plasma.[40] As the surface area to volume ratio is decreased in larger animals, the effective concentration of a key signaling molecule released at the site of injury (ie, the "first domino" in the hepatic effector cascade) would be diminished systemically. This possibility is consistent with the smaller degree of stimulation of System A activity and lack of System N induction in relatively larger animals (Fig 5), the effects of burn plasma on hepatic AIB accumulation just mentioned, and the direct correlation between liver damage, transporter stimulation, and size of the thermal injury.

These studies also demonstrate that alanine transport is more sensitive to activation by thermal injury than glutamine transport (Figs 1, 2, and 5). Different signaling mechanisms for each carrier could underlie this disparity, but the basis for this observation may be otherwise attributable to the significantly lower basal alanine transport rates compared with glutamine transport rates in whole liver[41] and in isolated hepatocytes (20 *v* 300 pmol · mg^{-1} protein · min^{-1}, respectively; Bode, Lohmann, Easson, and Souba, unpublished observations, March 1997). System A must therefore respond more dramatically than System N to support increased metabolic demand for its substrates. Although plasma membrane vesicles were used in these studies, the electrochemical driving force that differentially influences the activity of individual Na^+-dependent carriers is lost in the absence of intact cellular structure. Plasma membrane vesicles are ideal for assessment of the effects of pathologic states on individual transporters, but are not very useful for direct comparison of the inherent activity of distinct carriers in vivo, where other factors such as membrane potential and intracellular amino acid content collectively contribute to the observed transport rates. For example, initial-rate (50 μmol/L) Na^+-dependent transport velocities in HPMVs for both glutamine and alanine are each approximately 100 pmol · mg^{-1}

protein · 10 s^{-1} under the artificiallyimposed Na^+ gradient in our transport assays (Figs 1, 2, and 4); however, this is not the case in intact hepatocytes.

Based on results from the studies presented here, a model is proposed for the role of accelerated amino acid transport in the host metabolic response to burn injury. It is well established that the metabolic response to burn injury is biphasic.[5] The ebb phase occurs immediately after the injury and is characterized by decreased cardiac output, intravascular volume, and tissue perfusion. Thereafter, a transition is made to the flow phase characterized by increased metabolic rates, cardiac output, and erosion of lean body mass—a state that often persists until the convalescence of the patient. The transition between the ebb and flow phase in rats occurs approximately 12 hours after a 25% to 50% TBSA scald injury.[42] Since the observed inductions of System A and N activity occur in the context of hepatocellular damage, there exists a possibility that the transporters play a role in hepatic repair and regeneration. Indeed, similar temporal kinetics (at 24 hours) of System A induction have been reported during hepatic regeneration,[32,43] and evidence of hepatic DNA synthesis occurs 24 hours after burn injury.[1,2] Hepatic damage is evident early (8 hours), and the transient nature of System A and N induction at 24 hours postburn, coupled with the return of serum transaminase to control values by 72 hours, suggests that hepatic repair/regenerative processes may underlie the observed early changes in transporter activity. Stimulated transporter activity at 24 hours postburn may therefore reflect the collective effects of accelerated hepatic amino acid metabolism and regenerative/repair processes, while the smaller yet statistically significant increases in glutamine and alanine uptake rates that remain 72 hours postburn (Fig 2) may represent the support of accelerated hepatic gluconeogenesis that exists at this time.[44] Indeed, hepatic regeneration has been shown to peak at 24 hours posthepatectomy, with all regenerative processes complete by 72 hours.[45] The signals for hepatic regeneration during burn injury (eg, damage, necrosis, and inflammation) may be different from those after partial hepatectomy, but there is no reason to believe that the kinetics of the regeneration process per se differ between the two states. Although we have yet to study time points beyond 72 hours, other studies in both humans and rodents have shown that this period constitutes the flow phase characterized by accelerated metabolism.[5] The proposed model for the role of accelerated hepatic amino acid transport in burn injury is depicted schematically in Fig 7, and studies are currently being designed to further test this model.

In summary, the studies presented here indicate that burn injury stimulates hepatic glucogenic amino acid transport and that the relative contribution of systemic mediators in this process is partially distinct from those shown to play a role in other catabolic states such as endotoxemia and cancer. Based on metabolic studies in similar burn models, the temporal profiles of transporter activation coincide with hepatic regeneration/repair and heightened gluconeogenesis and ureagenesis. Augmented transporter activity may help to support the accelerated flux through such amino acid–dependent pathways. The transient nature of augmented transport rates reported here underscores the dynamic nature of adaptive hepatic physiology that occurs in response to the metabolic challenges posed by burn injury. Future studies will help to elucidate whether the activation of specific carriers is linked to individual metabolic pathways.

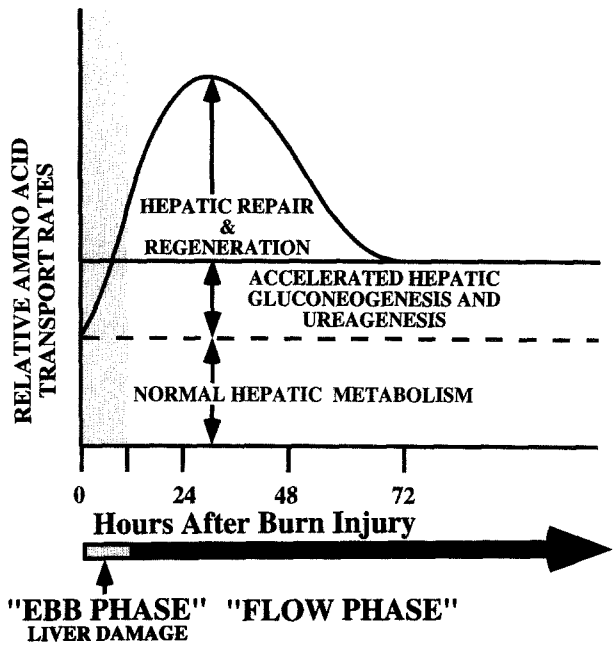

Fig 7. Proposed model for role of accelerated hepatic amino acid transport activity elicited by burn injury. Early scald-induced liver damage during the ebb phase of burn injury initiates (via as yet unidentified mediators) a regenerative/repair response including stimulation of hepatic amino acid transport. Subsequent burn-accelerated host metabolism occurs with the onset of the flow phase (approximately 12 hours postburn in rodents) and provides further metabolic/hormonal activation of transporter activity. By 72 hours, hepatic repair/regenerative processes are complete, and residual stimulation of amino acid transport activity supports augmented rates of gluconeogenesis for the burn wound repair process. The augmented hepatic amino acid transport activity at 24 hours is particularly pronounced in larger burns, consistent with greater degrees of liver damage in these animals.

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