Journal of Surgical Research **94,** 185–194 (2000)
doi:10.1006/jsre.2000.6006, available online at http://www.idealibrary.com on IDEAL®

RESEARCH REVIEW

Adhesion Molecules in Liver Ischemia and Reperfusion

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Submitted for publication April 19, 2000
Published online October 30, 2000

INTRODUCTION

Reperfusion of an acutely ischemic tissue is recognized to elicit an intense inflammatory response that causes tissue injury. The mechanisms of reperfusion injury are multifactorial, including all the biochemical and molecular events following ischemia. While restoration of blood flow is essential in ameliorating the progression of cellular injury associated with decreased oxygen and nutrient delivery, it is also followed by a series of complex reactions that paradoxically injures tissue [1–3]. Ischemic liver injury is a significant problem in clinical medicine that can occur as a consequence of circulatory shock or hepatic surgery (trauma, tumor resection, or liver transplantation) and may lead to local and organ systemic dysfunction. The purpose of this review is to analyze all important changes associated with the response of cell adhesion molecules (CAMs) after hepatic ischemia/reperfusion (I/R) injury.

PHASES OF LIVER ISCHEMIA/REPERFUSION

Interest in the better recognition of hepatic I/R injury has generated a dedicated effort to define the cellular and molecular cascades that underlie this response. There are two phases of hepatic injury following I/R: the initial phase (1–6 h postreperfusion) which is associated with the generation of toxic-free oxygen species, the activation of Kupffer cells, and an initial response of polymorphonuclear neutrophil (PMN) activation, and a later phase (6–24 h postreperfusion) associated with a rather intense state of PMNs influx to the liver and its subsequent biomolecular response [4–8]. Although both phases are recognized in the literature, they are not well circumscribed and they can readily interact and superimpose amid the overwhelming cytokine and chemokine characteristic reactions [3, 9–11]. This work, however, focuses its attention exclusively on the role of CAMs in liver I/R.

PMNs recruitment is known to be a multistep process, which involves initial contact and adhesion with the endothelium, transendothelial migration, and subsequent parenchymal cell adherence and damage. After I/R, CAMs are activated and/or upregulated on the surface of PMNs and endothelial cells. This activation and/or upregulation is induced by a variety of inflammatory molecules including lipid mediators, cytokines, chemokines, peptide chemoattractants, and nuclear transcription factors leading to the development of the adhesion cascade [9–12]. In the liver, the process of PMNs recruitment incorporates transmigration of neutrophils [13], due to the fact that not all CAMs have been identified in the liver sinusoidal vasculature, and some of the CAMs are mainly expressed in the hepatic arterioles or postsinusoidal venules [14–22]. A simplistic view of the changes occurring after I/R injury is schematized in Fig. 1.

CAMs are cell-surface glycoproteins involved in cell–cell and cell–matrix interactions. According to their sequence homology and individual structure, CAMs are divided into three major families: selectins, integrins, and the immunoglobulin superfamily (Fig. 2) [23].

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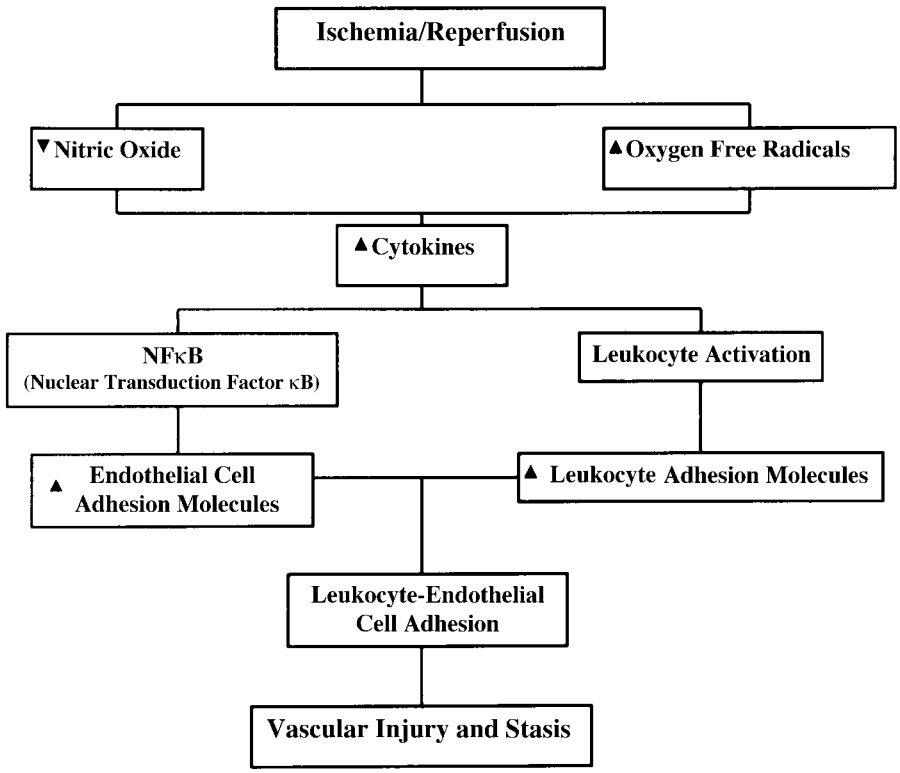

FIG. 1. A simplistic view of the pathophysiological mechanism involved in ischemia/reperfusion injury.

SELECTINS

The selectins are perhaps the most widely studied CAM family. They are lectin-domain glycoproteins with several complement binding repeats as the extracellular portion of the molecule [23] expressed on endothelial cells (P-selectin, CD62P, E-selectin, CD62E), platelets (P-selectin), and leukocytes (L-selectin, CD62L). They mediate the initial capture and support the rolling of PMNs on endothelial cells under physiological stress and they are necessary for the subsequent firm adhesion under conditions of flow [23, 24]. P-selectin is stored in the secretory α-granules of the platelets and the Weibel-Palade bodies of the endothelial cells [25]. Although P-selectin is not expressed in liver sinusoidal cells [14], cytokines can activate P-selectin expression in liver endothelial cells and postsinusoidal veins and then mobilize P-selectin to the cell surface leading to an initial transient expression [15]. E-selectin is not found in normal human liver tissue [16, 17] and its expression requires *de novo* protein synthesis. L-selectin is constitutively expressed on the surface of leukocytes and is shed from their cell surface following activation of the neutrophil with chemotactic factors (Fig. 3) [26].

Selectins bind to a variety of sialylated and fucosylated conjugates and express high affinity to a small subset of appropriately modified glycoproteins. The glycoprotein ligand with the most extensive characterized function is P-selectin glycoprotein ligand-1 (PSGL-1), a homodimeric mucin expressed on the surface of most leukocytes that binds P-selectin to an NH_2-terminal domain and E-selectin and L-selectin as well [27]. Thus, PSGL-1 is an essential ligand for primary tethering and rolling of neutrophils. All three selectins recognize sialyl Lewis^x (sLe^x) expressed on the neutrophil surface and similar carbohydrates as ligands [28, 29]. Binding of these selectins to their carbohydrate ligands initiates leukocyte infiltration and subsequent tissue damage [30, 31].

The expression of P-selectin in liver tissue was first demonstrated by Sanders and associates [32]. P-selectin has a biphasic response after hepatic I/R. The first peak occurs at 20 min after reperfusion and the second peak at 5 h of reperfusion [33, 34]. In this way, P-selectin plays a major role in the earliest phase of rolling and adherence of leukocytes in the hepatic microvasculature after I/R [35, 36] and also mediates reperfusion injury through platelet sequestration [37]. Although E-selectin has scarcely been studied in the hepatic I/R phenomenon, its maximum expression during liver I/R injury is noted at 5 h after reperfusion with a return to baseline levels by 24 h [38], contributing to the recruitment, adherence, and migration of PMNs. L-selectin has been found to mediate neutrophil

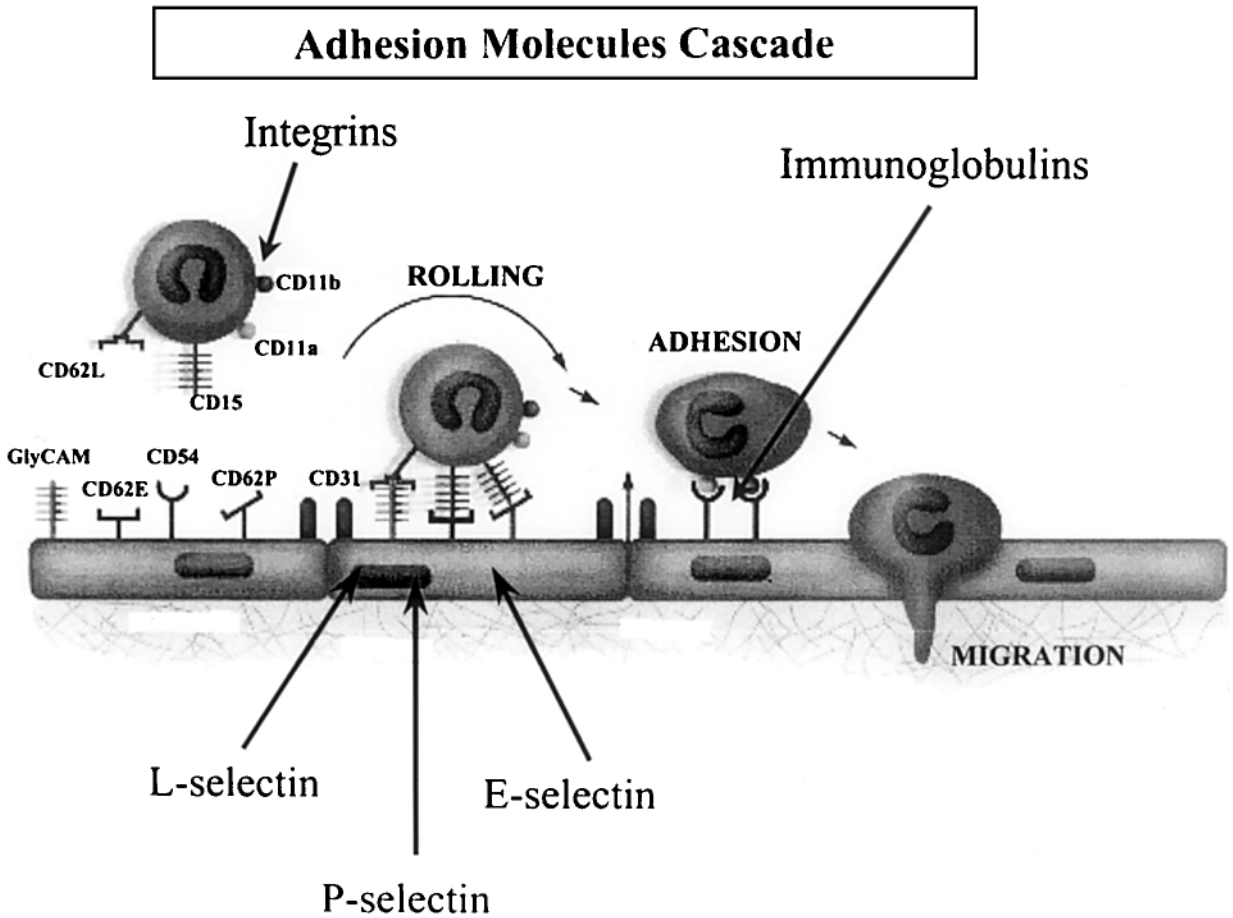

FIG. 2. Sequential activation and engagement of all the families of adhesion molecules resulting in PMNs migration into tissue (courtesy of J. M. Palma-Vargas, M.D.).

adhesion to liver I/R [39]; however, its expression pattern under these conditions has not been described.

Different strategies have been particularly relevant for ameliorating neutrophil-mediated hepatocellular damage following I/R by blocking selectin-mediated leukocyte–endothelial cell interaction. The first strategy was the development of a synthetic monoclonal antibody (Mab) directed against single members of the selectin family [40]. Garcia-Criado *et al.* [35, 41] identified beneficial effects using a Mab to P-selectin in a rat model of total hepatic ischemia given 30 min before reperfusion. An improved survival and a significant reduction of myeloperoxidase (MPO) activity in liver tissue were observed, as well as a protective effect of the tests that demonstrate hepatic injury [35, 41]. Other investigators [33, 36, 37] have been able to corroborate these results. Singh *et al.* [33] found an 8-fold increase in liver injury tests 10 h following I/R with significantly lower values in P-selectin-deficient mice. Yadav *et al.* [37] confirmed their findings utilizing P-selectin-deficient mice and showed an improved survival in the knockout animals when compared with controls [24, 27, 28]. In regards to L-selectin, Yadav

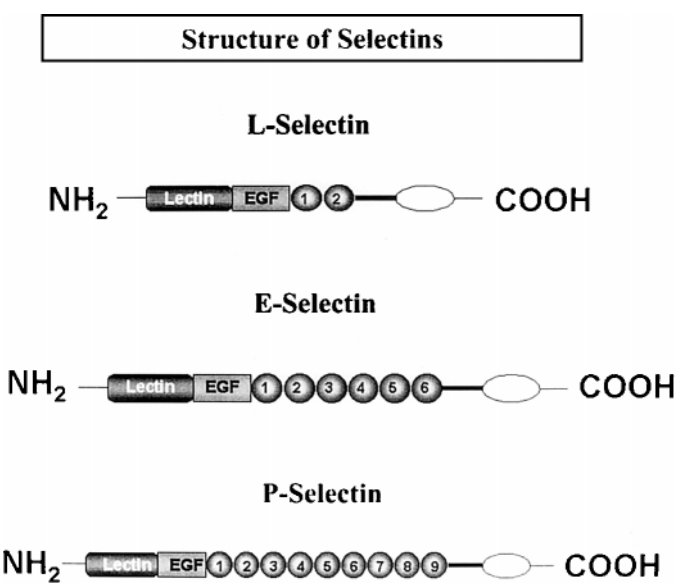

FIG. 3. Chemical structure of the three known selectin adhesion molecules (courtesy of J. M. Palma-Vargas, M.D.).

TABLE 1

Selective Review of the Literature Pertaining to the Selectin Family of Adhesion Molecules in Liver I/R Injury

Authors (Ref.)	Selectin family adhesion molecule	Animal type and time of ischemia	Treatment	Results
Garcia-Criado *et al.*, 1995 [35]	P-selectin	Rat, total 90 min	Mab[a] 30 min prior reperfusion	Liver injury protective effect Increased survival
Zibari *et al.*, 1998 [36]	P-selectin	Mice, partial 20 min	Mab[a] P-selectin-deficient animal	Liver injury protective effect Expression of P-selectin determined
Yadav *et al.*, 1999 [37]	P-selectin	Mice Partial, total 75–90 min	P-selectin-deficient animal	Liver injury protective effect Increased survival Reduction in platelet sequestration
Yadav *et al.*, 1998 [39]	L-selectin	Mice Partial, total 75–90 min	L-selectin-deficient animal	Liver injury protective effect Increased survival Reduction in leukocyte adhesion
Dulkanchainun *et al.*, 1998 [42]	P-, E-, L-selectin	Rat, total 45 min	Soluble PSGL-1[b] at ischemia and reperfusion	Liver injury protective effect Increased survival of isograft
Misawa *et al.*, 1996 [43]	P-, E-, L-selectin	Rat, total 90 min	Exogenous sLe^{x}[c] 5 min prior or at reperfusion	Liver injury protective effect Decreased myeloperoxidase activity
Palma-Vargas *et al.*, 1997 [45]	P-, E-, L-selectin	Rat, total 90 min	Small-molecule selectin inhibitor	Liver injury protective effect Increased survival

[a] Monoclonal antibody.
[b] P-selectin glycoprotein ligand-1.
[c] Sialyl Lewis^{x}.

and associates [39] recently showed a lesser decrease in PMNs adhesion and infiltration with a subsequent protective effect of the liver I/R injury using L-selectin-deficient mice. They found liver enzyme levels significantly reduced in mutant L-selectin-deficient mice versus wild-type mice. Survival in the mutant L-selectin mice was also increased compared to the wild-type mice group [39].

Various investigators have directed their attention to the use of ligands to P- and other selectins. Dulkanchainun and associates [42] used soluble PSGL-1 at the time of hepatic inflow occlusion in a warm model of liver ischemia. PSGL-1 afforded considerable protection from I/R injury, as demonstrated by decreased liver enzyme levels, histologic hepatic damage, and suppressed neutrophil infiltration. In the same study, soluble PSGL-1 was given before cold ischemic storage and showed a reduced degree of liver enzyme release and neutrophil infiltration. Furthermore, PSGL-1 administrated at the time of reperfusion of the transplanted liver also showed improved survival [42]. Using a competitive binding exogenous sLe^{x} oligosaccharide analog ligand, Misawa *et al.* [43] demonstrated a significant improvement in the protection of biochemical hepatic injury tests, reduced MPO, and PMNs subsequent migration into tissue after hepatic ischemia and reperfusion [43]. In addition, Garcia-Criado *et al.* [44], administering a glycomimetic sulfo-derivative of sLe^{x}, were able to show an increased survival and protective effect in an *in vivo* model of severe liver ischemia-reperfusion by decreasing selectin-mediated neutrophil migration. More recently, the effects of a novel small-molecule, nonoligosaccharide inhibitor of P-, E-, and L-selectin in the liver inflammatory response after ischemia–reperfusion were investigated by Palma-Vargas *et al.* [45]. Results from this study evidenced a beneficial effect in livers by decreasing PMNs infiltration and tissue damage when the compound was administered 15 min before reperfusion [45].

A selective summary of experiments dealing with strategies to modify the selectin family of cell adhesion molecules is summarized in Table 1.

INTEGRINS

Members of the integrin family of CAMs are membrane glycoproteins consisting of $\alpha\beta$ heterodimers with characteristic structural motifs [46]. Currently, there are six subgroups of integrins known, each with a common β-subunit (β_1–β_6) [29]. Resident liver cells, such as Kupffer cells, as well as newly recruited monocytes, T-lymphocytes, and neutrophils have β_1 and β_2 integrins on their cell surface [18]. The integrins involved in the leukocyte–endothelial cell adhesion are $\alpha_4\beta_2$ (very late antigen-4, VLA-4, CD49d/CD29), $\alpha_L\beta_2$

(lymphocyte functional antigen-1, LFA-1 or CD11a/CD18), $\alpha_m\beta_2$ (Mac-1, CD11b/CD18), $\alpha_x\beta_2$ (CD11c/CD18), and $\alpha_4\beta_7$ [26]. Most of the integrins have receptors that are stored in the neutrophil's peroxidase-negative granules [47]. The integrins are generally in a low avidity state, but they are activated to a high avidity conformation by various inflammatory mediators. Upon stimulation with several chemotactic factors, these neutrophil granules fuse with the cell membrane and increase integrins expression within minutes without *de novo* protein synthesis. Cell activation can result in conformational changes in the adhesion molecule, exposing their ligand-binding sites [48], leading to an integrin-mediated firm adhesion process and extravascular migration of PMNs [23, 29, 46]. The integrins conformational changes, specifically in LFA-1 and Mac-1, are suggested by Mab that react with these integrins only after cellular activation take place [49, 50]. The integrins adhesiveness can be rapidly regulated by the cells on which they are expressed. The transitory activation of their adhesiveness provides a mechanism for adhesion and, perhaps, from retraction of the trailing edge of a leukocyte from the substrate during cell migration [51]. Mature PMNs do not express $\alpha_4\beta_1$ or $\alpha_4\beta_7$, and their adherence is dependent primarily upon β_2 integrins [26].

The activation and inhibition of β_2 integrins, especially Mac-1, have been observed in several pathophysiological situations relevant to liver injury *in vivo*. Increased β_2 integrins expression was shown in the hepatic sinusoids and postsinusoidal venules during endotoxemia and sepsis [52–55]. Functional inhibition using a Mab to β_2 integrins in the liver after hemorrhagic shock was demonstrated by Anaya-Prado *et al.* [56] with significant protective effects in the liver.

Jaeschke and associates [55] used a CD11b-Mab to β_2 integrins Mac-1 (CD11b/CD18) in a liver I/R injury model. They showed a significant attenuated liver injury and reduced number of PMNs in the postischemic liver by pretreatment with the CD11b-Mab. Selective treatment with the CD11b-Mab only during reperfusion was similarly effective in this study also [47]. Palma-Vargas *et al.* [57] expanded this knowledge by blocking β_2 and β_3 integrins using individual and combined targeted Mabs against integrins. All Mab-treated groups demonstrated significant improvement in survival with improved liver function tests and histological damage scores in the Mab-treated groups as compared to controls. Fibrinogen consumption as a marker of activation of the coagulation cascade was significantly decreased for the combined anti-β_2 and β_3 integrin-treated group and the individual anti β_3 integrins [57].

From these multiple studies, it is clear that β_2 integrins play a pivotal role in PMN-mediated I/R injury. They mediated firm adhesion of PMNs to the vascular wall, allowing diapedesis, migration, and adhesiveness of PMNs into liver tissue.

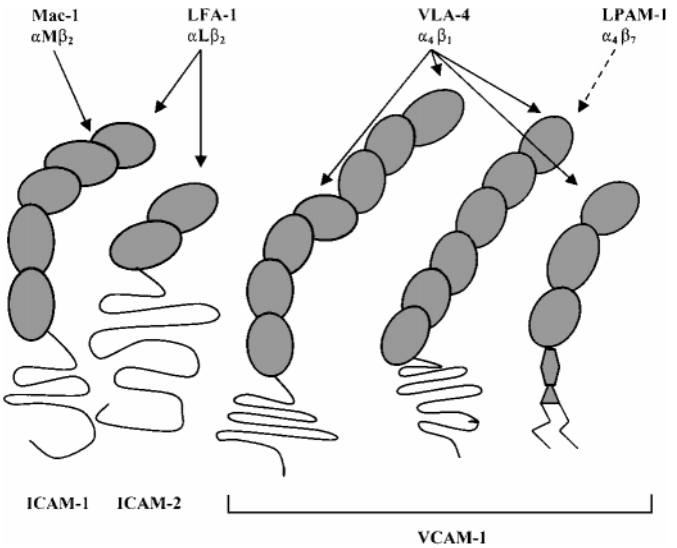

FIG. 4. An example of various members of the immunoglobulin superfamily receptor on endothelium and their integrin-binding sites.

IMMUNOGLOBULIN SUPERFAMILY

When the initial association was established with ICAM-1 binding to LFA-1, several different immunoglobulin superfamily members were found to be expressed on endothelium and bind to integrins on leukocytes [29], as shown in Fig. 4. Intercellular adhesion molecules 1, 2, and 3 (ICAM-1, ICAM-2, ICAM-3), vascular cell adhesion molecule-1 (VCAM-1), and platelet endothelial cell adhesion molecule-1 (PECAM-1) are members of the immunoglobulin Ig-like family [29, 48]. ICAM-1, ICAM-2, and ICAM-3 are products of distinct and homologous genes and were initially identified by their ability to interact with β_2 integrins [58–60]. Induction of ICAM-1 on endothelium and other cells by inflammatory cytokines cause ICAM-1–β_2 integrin interactions to promote firm adhesion to endothelial cells and may increase leukocyte extravasation at inflammatory sites, where the constitutive expression of ICAM-2 may be important for leukocyte trafficking [29, 51]. VCAM-1 is inducible by cytokines on endothelial cells and on more nonvascular cells than ICAM-1 [61]. VCAM-1 is a ligand for VLA-4 integrin and binds weakly to $\alpha_4\beta_7$ [62]. PECAM-1 is involved in the diapedesis of PMNs through the endothelial junctions (Fig. 5) [26].

ICAM-2, ICAM-3, and PECAM-1 are constitutively expressed on vascular lining cells [19–21]. PECAM-1 expression cannot be induced by cytokines [63]. Low constitutive expression of ICAM-1 can be found on the hepatic endothelium and Kupffer cell in normal liver

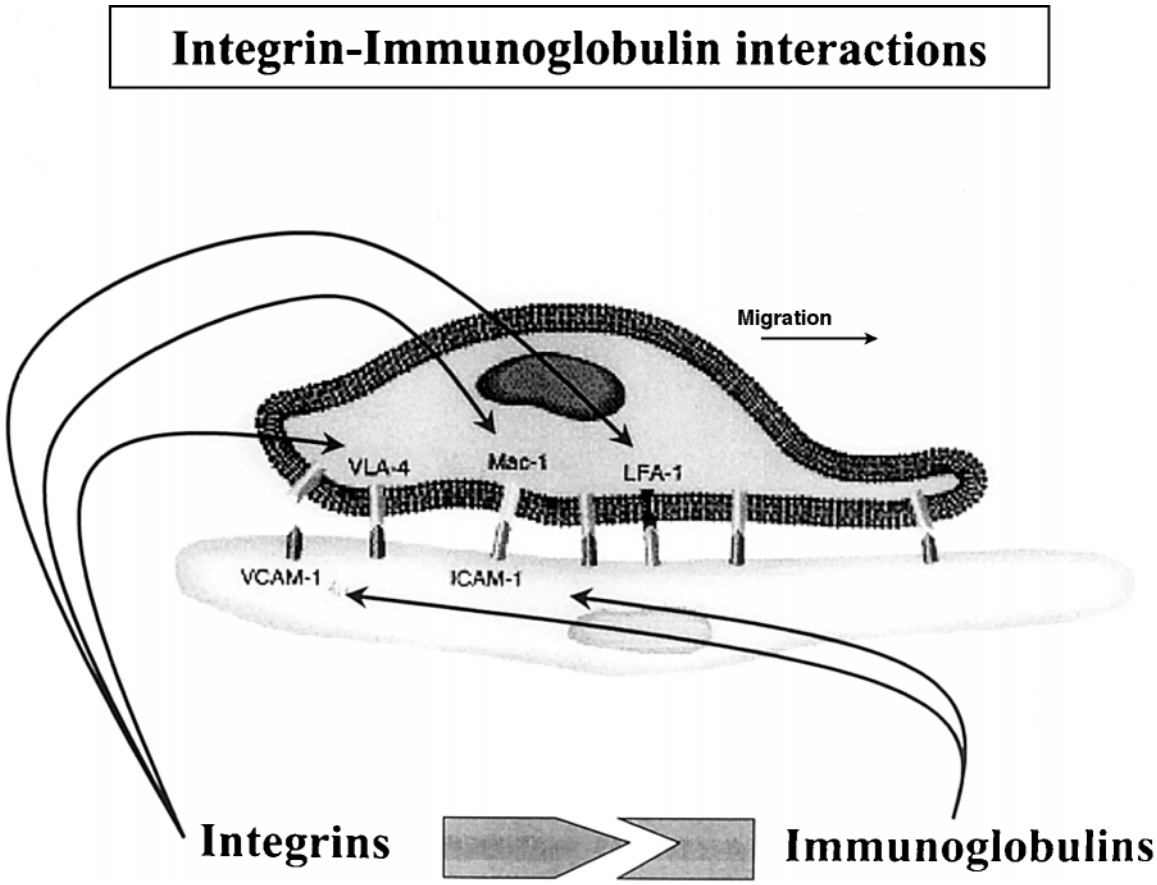

FIG. 5. Binding of leukocytes and integrins with endothelial immunoglobulin in the process of PMNs migration (courtesy of J. M. Palma-Vargas, M.D.).

tissue. Although no ICAM-1 is detected in hepatocytes, ICAM-1 is upregulated by cytokines on virtually all liver cells [19, 20]. There is low VCAM-1 expression on normal liver endothelial cells and the upregulation of this Ig-like CAMs is induced also by different cytokines [22].

Blocking Ig-like CAMs family has also been a successful therapeutic strategy for attenuating liver injury in a number of different studies of hepatic I/R. Data published by Suzuki *et al.* [64] demonstrated a higher significant liver enzyme activity in plasma at 6 h after total hepatic ischemia/reperfusion in the control group compared to the Mab to ICAM-1-treated group. Mab to ICAM-1 inhibited PMNs infiltration and hepatic necrosis when compared with the control group. Survival was also significantly increased using the Mab to ICAM-1 [59]. Similar findings provided by Vollmar and associates [65] supported *in vivo* evidence for the function of ICAM-1 during liver I/R injury. They investigated the role of Mab to ICAM-1 using intravital fluorescence microscopy in a rat model of partial I/R. The treatment with Mab to ICAM-1 effectively inhibited postischemic leukocyte adherence and hepatic reperfusion injury measured by sinusoidal perfusion and the excretory function and hepatocellular integrity was also significantly ameliorated [65].

Based on this previous evidence, Marubayashi and associates [66–68] were able to combine different Mabs directed to ICAM-1 and its ligand, the β_2 integrins. They showed a beneficial effect of treatment with these Mabs observed at 6, 12, and 24 h after reperfusion reflected in a suppression of PMNs infiltration, an enhanced recovery of hepatic adenosine triphospate, and a diminished serum alanine aminotransferase levels after reperfusion [67, 68]. Nakano *et al.* [69] using a different approach investigated the efficacy of the intraportal injection of the same Mab to ICAM-1 used by Suzuki [64]. He demonstrated a histologically suppressed liver cell damage with the intraportal administration of this Mab after 60 min of warm ischemia [69]. More recently, Yadav *et al.* [39] introduced the use of a ICAM-1-deficient mouse, combined with a L-selectin-deficient mouse, to test the liver I/R injury in these animals. They reported significantly reduced liver enzyme levels, diminished leukocyte adhesion, and better survival in the mutant mice group after partial hepatic ischemia [39].

CLINICAL SIGNIFICANCE IN HEPATIC SURGICAL DISEASES

The pathological presence of neutrophils in the liver has been demonstrated under a variety of clinical conditions. There are a different number of hepatic diseases that are predominantly involved in I/R mechanisms of injury, mainly related to their surgical treatment (hepatectomy or liver transplantation). For most acute and chronic hepatic diseases there have been reports of increased plasma levels of soluble forms of P- and E-selectin, ICAM-1, and VCAM-1 (sCAMs) [70]. The formation of alternatively spliced forms of these proteins has been postulated as the main source of these sCAMs [70]. Expression of sICAM-1, sVCAM-1, and sE-selectin is increased on interlobular bile ducts, proliferating ductules, and periseptal hepatocytes in patients with primary biliary cirrhosis and primary sclerosing cholangitis [71–73]. Significant elevation of sICAM-1 in patients with hepatolithiasis before surgery compared to normal subjects has been found [74] and liver metastases are markedly enhanced in ICAM-1 and LFA-1 homozygous deletionally mutant (gene knockout) mice after 2 weeks of B16 melanoma cell injections [75, 76].

Shimada and associates [77] intended to clarify the role of CAMs in hepatic resection by studying the expression of CAMs and the measurement of their soluble fractions in blood. They obtained liver biopsies before and after hepatectomy and samples from hepatic venous blood flow. They were able to demonstrate an enhanced expression of either ICAM-1 or VCAM-1 with more than 40 min of total hepatic surgical time compared to surgical procedures with less than 40 min of total ischemic time. They found a higher incidence of postoperative complications in the positive ICAM-1 group compared to the negative ICAM-1 group. They also found that the soluble fractions of ICAM-1 and VCAM-1 were higher in cirrhotic patients than in noncirrhotic patients and correlated with the preoperative levels of albumin and liver enzymes and diminished after hepatectomy [77].

Liver transplantation is inevitably subjected to warm and cold ischemia. Clinical consequences of I/R injury conditioned by cold and/or warm ischemia are initial poor function in 15–30% of patients [78] or even primary nonfunction of a graft that has functioned well in the donor. The incidence of primary nonfunction sometimes requires retransplantation and it is associated with devastating consequences of individual patients. Recent studies have explored the expression of CAMs and their possible role as a reliable markers for graft functioning, prognosis, and ultimate clinical outcome after liver transplantation either in experimental animal models or in humans [78–82]. Nishimura and associates [79] studied the ICAM-1 expression and the use of a $F(ab')_2$ fragment of a Mab to ICAM-1 in an animal model of liver transplantation. They showed a strong ICAM-1 expression after reperfusion in animals that received livers stored for 6 h compared to animals that received livers stored for 1 h. The liver enzyme levels were significantly higher and correlated well with the 6-h storage group. When the liver enzyme levels were markedly reduced, they were associated with a reduced accumulation of leukocytes in the liver after treatment with the $F(ab')_2$ fragment of the Mab to ICAM-1.

Mueller and associates [78] studied the release of different cytokines, soluble CAMs, and extracellular matrix parameters during and after reperfusion in 81 patients with 85 liver transplants. They were able to demonstrate a significant correlation between higher levels of sVCAM-1 and sE-selectin with the length of preservation and reperfusion injury. Furthermore, sE-selectin levels during reperfusion were predictive of subsequent development of acute allograft rejection [78]. Kiuchi and associates [80] evaluated semiquantitatively P-selectin, β_2 integrins, and ICAM-1 tissue expression from liver graft biopsies at the end of cold preservation and 2 h after reperfusion using specifically directed Mabs. They found an increased expression of P-selectin and β_2 integrins after reperfusion in correlation with prereperfusion levels of expression. P-selectin, β_2 integrins, and ICAM-1 expression were also correlated with peak liver enzyme measurements and steatosis after reperfusion. β_2 integrins and ICAM-1 expression were associated with primary graft nonfunction and ICAM-1 with early acute rejection [80].

In contrast, Viebhan [81] found no relationship between reperfusion injury, cold ischemic time, and sICAM-1 in a series of 75 consecutive liver transplants, but a strong correlation between the course of sICAM-1 release and the degree of reperfusion injury was found.

Finally, Thiel and associates suggested the use of β_2 integrins and L-selectin as a useful predictor of early graft dysfunction [82]. They conducted a study focusing on the expression of CAMs on PMNs to monitor PMNs activation and the relationship of PMNs activation and parameters of hepatocellular integrity in a series of 20 patients that underwent orthotopic liver transplantation. They classified the patients as a responder if the expression of one or both classes of adhesion molecules determined before reperfusion was changed at least to the extent expected to occur by chance in less than 5% of the cases. Eight of 20 patients were classified as responders. They showed a significantly higher postoperative liver enzyme activity than the nonresponder patients, indicating that PMNs activation was associated with a greater hepatocellular damage [82].

FUTURE TRENDS

We described the significant advances noted in the important role that the adhesion molecules play in liver I/R. New molecular pathogenic mechanisms have been deciphered in the experimental therapeutic implications associated with the use of these molecules. The future relies now on our ability to develop compounds that can be readily manufactured and utilized without untoward effects and still be potent inhibitors of adhesion molecules.

We expect four technologies to occupy our attention in the present or near future: first, the development of small-molecule selectin inhibitors based on combinatorial chemistry through a computerized carbohydrate model, currently being used experimentally; second, the building of antisense oligonucleotides by identifying target molecules, such as ICAM, of use under experimental conditions also; third, the development of haptemers to generate compounds of antiadhesion molecules properties; and, fourth, and the most distant target yet to be obtained, the use of specific gene therapy directed against the adhesion molecule cascade.

The ideal compound will be one that is easy to make, inexpensive, and highly effective against the target identified. We believe that the blockade of one adhesion molecule will not be sufficient and that the inhibition of several of them will be required to reach a maximal effect. The identification of various signaling pathways, that potentially have negative implications, will offer another way of improving our therapeutic ability.

In summary, this field is young and awaits the recognition of new and advanced mechanisms for a better understanding and treatment of the ischemic and reperfusion injury.

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