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Expression of cell-matrix molecules and integrin receptors in human liver grafts during chronic rejection

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Abstract The inflammatory response of immune cells to target cells and cell-matrix molecules is regulated by several receptor-ligand molecules. As fibrosis develops in ongoing chronic rejection after liver transplantation, it is of interest to analyze patterns of integrin receptors and cell-matrix molecules in order to study the relation between immune cells and the stromal and parenchymal cells. In the present study, we demonstrated the expression of these molecules in chronic rejected human liver grafts using immunohistochemical techniques. The results showed a differential expression and induction of integrin receptors and cell-matrix molecules on resident liver cells, especially on sinusoids, reflecting a state of chronic inflammation and a specific interaction between integrin receptors and cell-matrix molecules. The patterns of induced integrin receptors on graft-infiltrating cells was closely related to the local production of cell-matrix molecules and reflected the final sequence of a stepwise progress of the inflammatory reaction.

Key words Integrin receptor
Cell-matrix molecule · Liver transplantation · chronic rejection

Introduction

The inflammatory response is regulated by a network of cytokines and intercellular interactions in various pathological stimuli. It has been found that a number of cell membrane receptors to cell-matrix molecules called integrin receptors bind to a variety of cell-matrix molecules [16]. They probably take part in inflammatory tissue reactions and the interstitial infiltration of lymphocytes during tissue inflammation. In liver transplantation, long-term graft performance is affected by chronic graft rejection [18]. However, we do not have a sufficient understanding of the causes and mechanisms of chronic graft dysfunction. The present study was undertaken to determine the local expression of integrin receptors and cell-matrix molecules on resident and infiltrating cells in chronic rejected liver grafts in comparison to normal livers.

Materials and methods

Tissue sampling

Fifteen liver specimens diagnosed as chronic rejecting liver grafts were obtained at the time of retransplantation. Normal liver specimens were obtained from four liver resections at least 3 cm distant from tumor nodules and three cold perfused livers before transplantation that were surplus donor tissue of split liver grafts for pediatric patients. All samples were frozen in liquid nitrogen and then stored in a deep freezer at − 80 EC. For immunohistochemical analysis, cryostat sections of 4 μm thickness were obtained.

Immunohistological staining

The primary antibodies used in this study were monoclonal antibodies for VLA (very late antigen)-1 (TS2/7, purchased from T-cell Science, Cambridge Mass., USA), VLA-2 (Gi9), VLS-3 (P1B5), VLA-4 (HP2/1), VLA-5 (SAM-1), VLA-6 (GoH3), CD51 (AMF/7; AMF/7 and monoclonal antibodies for VLA-2 to VLA-6 and CD51 were purchased from Dianova, Hamburg, Germany), fibronectin (clone II from GIBCO BRL, Berlin, Germany), tenascin (TN2), collagen VI (F33), and laminin-A chain (monoclonal antibody 19.24). The latter three mouse monoclonal antibodies were kindly supplied by D. Schuppan, Klinikum Steglitz Free University, Berlin, Germany. The expression of the integrin receptors and the cell-matrix molecules was studied using standard immune peroxidase immunohistological techniques [15].

Semiquantitative evaluation

The expression of the integrin receptors and the cell-matrix molecules were evaluated by a semiquantitative scale running from minus (−) to plus 2(++) or 3(+++) according to the intensity of staining on resident liver cells and the number of positive leukocytes and monocytes (graft-infiltrating cells), Kupffer cells, and portal interstitial cells, respectively.

Results

The expression of integrin receptors and cell-matrix molecules on the resident liver cells in normal and chronic rejected livers is shown in Table 1.

Normal liver

All of the molecules and integrin receptors except for VLA-2 and VLA-4 were found on the portal vascular endothelium. VLA-1, VLA-5, CD51 and all the cell-matrix molecules except for laminin were found along the sinusoidal endothelium. VLA-1 and VLA-5 were found on the hepatocytes, VLA-2, -3, -6, and CD51 were found on the bile ducts, and all the cell-matrix molecules were found at the base of biliary epithelial cells. VLA-1, -4, -5, and CD51 were found on the portal interstitial cell/Kupffer cell.

Chronic rejected liver

The expression pattern on the portal vascular endothelium was the same as in normal liver. Strong expression of the integrin receptors and cell-matrix molecules were found on the sinus endothelium (VLA-2 was only partly positive). On the central veins, all cell-matrix molecules were positive but laminin was negative in four cases in which prominent fibrotic changes were found around central veins. VLA-1, -5, -6, CD51, and fibronectin were observed on the hepatocytes. VLA-2, -3, -6, and CD51 were present on the bile ducts. Portal interstitial cells and kupffer cells showed a broad positivity for most integrin receptors except for VLA-2. In addition,

Table 1 Expression of the integrin receptors and extracellular matrix on resident liver cells. For the expression in chronic rejection of allografted livers, only changes compared to normal liver are shown. Empty space represent the same expressions as in normal liver (*N* normal livers, *C* chronic rejected grafts)

	Endothelium						Epithelium			
	Portal vascular		Central vein		Sinusoids		Hepatocytes		Bile duct	
	N	C	N	C	N	C	N	C	N	C
Integrin receptors										
VLA-1	+		+	+/++	+	++	±/+	+	−	
VLA-2	−	−/+	−		−	−/+[a]	−		+	
VLA-3	+		−		−	±+[a]	−		+	
VLA-4	−		−		−	+[a]	−		−	
VLA-5	+		+		+	++	±/+		−/+	
VLA-6	+		−		−/+	+[a]	−/±	+	+	
CD51	+		−/+	+	+	+/++	−/±	−/+	+	
Cell-matrix molecules										
Fibronectin	+		++	++/+++	+	++/+++	−	−/±	+	
Tenascin	−/+	+	−/±	+	±	+/++[a]	−		−/±	−/+
Collagen VI	+		±/+	+/++	±/+	+	−		+	
Laminin	+		−/+	+	−[b]	+/++[a]	−		+	+/++

[a] Preferentially stained on connective tissue-epithelial interface and aciner zone I (I > III > II)

[b] Positive staining was found weakly only at the connective tissue-epithelial interface

Table 2 The positive expressions on graft-infiltrating cells and surrounding matrix in chronic rejected liver grafts

Integrin receptors on graft-infiltrating cells		Cell-matrix molecules around graft-infiltrating cells	
VLA-4	++	Fibronectin	++
VLA-5	+	Tenascin	+/++
VLA-6	±/+	Collagen VI	+
CD51	+		

VLA-3, and VLA-6 were partly induced on Kupffer cells and VLA-6 was partly induced on portal interstitial cells.

There was an enhanced expression of VLA-4 on the graft-infiltrating cells was in the portal tract and central vein area in all grafts (Table 2). VLA-5-, VLA-6- and CD51-positive cells were partly found. A major proportion of the intima-adherent leukocytes expressed VLA-4. Intravascular VLA-5- and CD51-positive leukocytes were also partly found. Fibronectin and collagen stained the full septal thickness with equal intensity. In contrast, tenascin was expressed mainly in perivascular areas and in the peripheral part of the portal area. These molecules expressed strong positivity around graft-infiltrating cells (Table 2). Laminin showed no or only weak expression on connective tissue.

Discussion

In the regulation of tissue inflammation and repair mechanisms, the processes of intravascular adhesion, transmigration, and infiltration by leukocytes and platelets is mainly mediated by receptor-ligand interactions with target cells (cell-cell) and extracellular matrix proteins (cell-matrix) [16]. From our results, it is remarkable that all intravascular adherent leukocytes and all graft-infiltrating cells expressed VLA-4. It has been shown that VLA-4 mediates adhesion of lymphocytes to activated endothelial cells by binding to an inducible endothelial cell surface protein called VCAM-1 (vascular cell adhesion molecule-1 [4]. The sites on VLA-4 involved in VCAM-1 binding are distinct from those involved in fibronectin binding. This can be suspected to play a key role in the process of definitive adhesion, emigration, and tissue infiltration. From the viewpoint of the deposition of cell-matrix molecules, a strong expression around graft-infiltrating cells in connective tissue was found, especially prominent in fibronectin, tenascin, and collagen VI. Tenascin and identical molecules can bind to fibronectin and to proteoglycans [10, 17]. Tenascin contains an RGD (arg-gly-asp) sequence and can mediate cell attachment through an integrin-type receptor [3].

Portal vascular endothelial cells and sinusoidal lining cells showed a broad positivity and inducibility for most integrin receptors and cell-matrix molecules. With regard to sinusoids, VLA-1, VLA-5, CD51, fibronectin, and collagen VI were found in normal liver. In rejected liver, all the integrin receptors and cell-matrix molecules were strongly expressed, i.e., the expression of fibronectin and its receptors, VLA-3, -4, -5, and CD51 were induced and the expression of laminin and its receptors, VLA-1, -2, -3, and -6 were also induced in sinusoidal lining cells in inflamed liver. Laminin is not detected in sinusoids in normal liver, where a basement membrane is not detectable by electron microscopy [2, 9]. On the other hand, sinusoids acquire laminin in pathological conditions [2, 9]. Electron microscopy demonstrates typical basement membranes called capillarization of the sinusoid [2, 14]. In our results, strong expression of all the cell-matrix molecules was observed on sinusoids in chronic rejected liver grafts by light microscopy and seems likely to correspond to the formation of visible basement membrane (sinusoidal capillarization). Pericellular collagen and basement membrane deposition, mainly observed in areas of sinusoidal capillarization, may lead to increased resistance to plasma exchange between blood vessels and liver cells. This mechanism may be one of the causative factors for chronic liver dysfunction in chronic rejected liver grafts.

Hepatocytes showed a more restricted expression of cell-matrix receptor molecules. With regard to the expression of cell-matrix molecules, positive staining of fibronectin was partly found in chronic rejected livers in our study. However, it has been reported that hepatocytes seem to contribute only a minor part to cell-matrix molecule production [12]. It is now evident that retinoid-storing cells (fat-storing cells, parasinusoidal cells, Ito cells), which are localized in the space of Dissé, are the most important connective tissue producing cell type in inflammatory disease [6, 8]. From the viewpoint of the function of retinoid-storing cells, an activation and transformation of retinoid-storing cells is mediated by paracrine and autocrine loops involving transforming growth factor (TGF) beta and platelet-derived growth factor (PDGF), especially in the fibrogenic phase [5, 11]. This transformed retinoid-storing cell (myofibroblast-like cell, transitional cell) secrets many kinds of cell-matrix molecules and cytokines [7]. It seems likely that the effects of cytokines on the expression of integrin receptors, the activation of interstitial cells and retinoid-storing cells, as well as the production of matrix molecules, form a circuit of inflammatory fibrogenesis. Considering that VLA-4, VLA-5, and CD51 were positive on

graft-infiltrating cells in our study and that these integrin receptors have a binding site at least to fibronectin, we suggest that the patterns of induced integrin receptors on graft-infiltrating cells relate to the local production of cell-matrix molecules, reflecting a vicious circle in the chronic inflammatory response that facilitates migration of graft-infiltrating cells. Our data also showed a strong expression of integrin receptors and extracellular matrix on sinusoids in chronic rejected grafts. We suggest that these findings reflect a state of chronic inflammation and fibrogenesis.

The patterns of expression in chronic rejection of allografted livers are similar to those in chronic inflammatory liver disease that have been reported by some investigators [1, 13]. It seems that chronic inflammation once triggered by viral infections, autoimmune disease, or allogeneic response, may lead to a common pattern of ultimate inflammatory reaction, probably modified by specific allogeneic mechanisms.

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