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Modulation of ICAM-1 tissue expression in patients with liver transplantation (LT) and acute rejection (AR) after glucocorticoid treatment

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Abstract Acute rejection (AR) is a frequent complication following liver transplantation (LT). ICAM-1 may be involved in its pathogenesis. High doses of glucocorticoids are the standard treatment in these patients. The aim of this study was to describe corticoid effects on ICAM-1 tissue expression in liver biopsies of patients with LT and AR. The study included liver biopsies performed before and after treatment in 12 patients with LT and proven AR. In 10 patients AR was reversible and in 2, was steroid resistant. For immunohistochemistry, an indirect immunoperoxidase technique was used. Each histology section was semiquantitatively evaluated as follows: 0: < 10% staining, 1: 10–25%, 2: 25–50%, 3: > 50%. The control group comprised nine patients with LT and normal liver biopsies. In pre-treatment liver biopsy samples, ICAM-1 was markedly expressed on sinusoidal cells (2.41 ± 0.66), and there was also expression on periportal (0.66 ± 0.65) and perivenular hepatocytes (0.83 ± 0.57). By contrast, in the liver tissue from the control group, sinusoidal ICAM-1 reactivity was significantly lower (0.88 ± 0.33; $P < 0.05$), and hepatocytes showed no reliable ICAM-1 expression. After steroid treatment the intensity of ICAM-1 decreased significantly in sinusoids (1.5 ± 0.67; $P < 0.05$) and in perivenular hepatocytes (0.25 ± 0.86; $P < 0.05$). Additionally, we also observed a decreased ICAM-1 reactivity in portal hepatocytes (0.25 ± 0.62), but these differences did not reach statistical significance. Remarkably, after treatment, hepatocytes did not show ICAM-1 reactivity in resolved AR, but in corticoid-resistant patients AR did not change or increase. In conclusion, in patients with LT and AR, ICAM-1 was expressed in hepatocytes and with more intensity in sinusoid cells. Additionally, a down-regulation of the ICAM-1 tissue expression after corticoid treatment may exist, although in corticoid-resistant AR no modulation on ICAM-1 tissue expression was observed.

Key words Liver transplantation · Rejection · ICAM-1 · Immunohistochemistry

Introduction

Acute rejection (AR) can be defined as the inflammation of the allograft elicited by a genetic disparity between the donor and the recipient, primarily affecting interlobular bile ducts and vascular endothelia, including portal and hepatic veins and, occasionally, the hepatic artery and its branches [1]. Despite the introduction into clinical practice of new and potent immunosuppressive agents, AR occurs commonly and remains a major

cause of morbidity after liver transplantation (LT) [2]. Bolus intravenous corticosteroid therapy is the most commonly used treatment for episodes of cellular rejection [3]. Nearly 80% of cases of AR respond to a single course of high-dose corticosteroids [4].

The immune response in allograft liver transplantation is extremely complex and remains poorly understood. The induction of AR begins with host lymphocyte recognition of the donor antigens on the antigen-presenting cells [5]. Once recognition of allograft antigen occurs, transmembrane signals lead to intracellular activation, expansion and differentiation of the recipient's T lymphocytes [6]. The last step is the destruction of the allograft antigens. During AR several cytokines are secreted and some of them (interleukin-1, tumour necrosis factor α and γ-interferon) up-regulate the expression of adhesion molecules [7]. Adhesion molecules are really important in cell-cell interactions and in T-cell activation [8], and their induction during AR episodes has been previously described [9, 10]. ICAM-1 is an adhesion molecule from the immunoglobulin superfamily that is recognised to be an important factor in the multi-step process of the migration and adhesion of lymphocytes during AR [11]. Expression of ICAM-1 on hepatocytes has been demonstrated on biopsy histology during allograft rejection and viral infections [12]. In the present study, ICAM-1 tissue expression in patients with LT and AR was investigated. Additionally, we studied the effects of intravenous glucocorticoids on the ICAM-1 reactivity, analysing the differences between cortico-sensitive and cortico-resistant episodes.

Patients and methods

Patients

We studied 33 liver biopsies of 21 patients who received a liver transplantation. Of these patients, 12 presented with an AR episode and 9 patients constituted the control group. All of them received triple immunosuppression therapy (cyclosporin, azathioprine and prednisone). When AR was suspected clinically (transaminase, bilirubin, alkaline phosphatase levels rose without other causes of graft dysfunction) a liver biopsy (with Menghini needle by a percutaneous route) was performed to obtain histological confirmation. All episodes of AR were treated with high doses of intravenous glucocorticoids. After treatment a new biopsy was performed to assess the evolution of AR. In the control group, the biopsies were obtained within a similar period of time as patients with AR, but no clinical or histological features of AR, or any other graft dysfunction were observed. All patients gave informed consent to participate in this study.

Table 1 Global assessment of rejection grade mode

Global assessment	Criteria
Indeterminate	Portal inflammatory infiltrate that fails to meet the criteria for the diagnosis of acute rejection
Mild	Rejection infiltrate in a minority of the triads that is generally mild and confined within the portal spaces
Moderate	Rejection infiltrate, expanding most or all the triads
Severe	As above for moderate with spillover into periportal areas and moderate to severe perivenular inflammation that extends into the hepatic parenchyma and is associated with perivenular hepatocyte necrosis

Liver tissue studies

Liver histology

The same expert pathologist (E. A.) evaluated the liver biopsies. The number of portal tracts in each liver biopsy was analysed to assess the diagnostic efficacy of the liver samples. The severity of AR was graded according the grading system of Demetris [16] (Tables 1 and 2).

Liver immunohistochemistry

For the immunohistochemical analysis, 4-μ sections were cut from frozen liver tissue. These sections were fixed in acetone for 10 min and were kept in a refrigerator at -70 °C until the immunohistochemical study was performed. Firstly, the sections were refixed with chloroform for 15 min. Then they were incubated for 40 min with RR1/1 anti-ICAM-1. The sections were consequently incubated with peroxidase-conjugated rabbit anti-mouse inmunoglobulins and, finally, they were developed with the Graham-Karnovsky solution containing 0.5 mg/ml of 3'3 diaminobenzidine tetrahydrocholide (DAB; Sigma Chemical Co., St Louis, Missouri) and hydrogen peroxide. After each incubation, sections were washed with tris-buffered saline (TBS) isotonic buffer, pH 7.6. Sections were counterstained with Carazzi's haematoxylin, dehydrated and mounted by using routine methods.

Each liver section was semiquantitatively scored by the same observer in the following way: 0: < 10% positive staining cells, 1: 10–25%, 2: 25–50%, 3: > 50% positive staining. The data were averaged to median values configurating a numerical score for each liver biopsy specimen and used for statistical analysis The statistical analysis was performed with the R-Sigma Babel (Horus Hardware, Madrid). Wilconxon's test was used for no parametric data. A P value < 0.05 was considered significant.

Results

Clinical characteristics

Nine episodes of rejection were diagnosed in the first month after transplantation (14 ± 4.7 days) and only three appeared thereafter (108 ± 15.8 days). In two

Table 2 Rejection activity index (RAI). Total score: sum of the components

Category	Criteria	Score
Portal inflammation	Mostly lymphocytic inflammation involving, but not noticeably expanding a minority of the triads	1
	Expansion of most or all of the triads by a mixed infiltrate containing lymphocytes with occasional blasts, neutrophils and eosinophils	2
	Marked expansion of most or all of the triads by a mixed infiltrate containing numerous blasts and eosinophils with inflammatory spillover into the periportal parenchyma	3
Bile duct inflammatory damage	A minority of the ducts are cuffed and infiltrated by inflammatory cells and show only mild reactive changes such as increased nuclear/cytoplasmic ratio of the epithelial cells	1
	Most or all of the ducts infiltrated by inflammatory cells. More than an occasional duct shows degenerative changes such as nuclear pleomorphism, disordered polarity and cytoplasmic vacuolization of the epithelium	2
	As above for 2 with most or all of the ducts showing degenerative changes or focal luminal disruption	3
Venous endothelial inflammation	Subendothelial lymphocytic infiltration involving some, but not a majority of the portal and/or hepatic venules	1
	Subendothelial infiltration involving most or all of the portal and/or hepatic venules	2
	As above for 2, with moderate or severe perivenular inflammation that extends into the perivenular parenchyma and is associated with perivenular hepatocyte necrosis	3

Table 3 Values of the staining score in the different biopsy groups

	ICAM-1 Pretreatment	ICAM-1 Postreatment	Control group
Sinusoids	2.41 ± 0.66	1.5 ± 0.67	0.88 ± 0.33
Perivenular Hepatocytes	0.83 ± 0.57	0.25 ± 0.86	0
Periportal Hepatocytes	0.66 ± 0.65	0.26 ± 0.62	0

cases these late rejection episodes were related to vomiting and diarrhoea. The clinical symptoms were very weak: only two patients presented with fever (without concomitant infectious disease). In ten patients the episodes of rejection responded to corticoid treatment, and in two cases the episodes of rejection were cortico-resistant (no improvement in either clinical or histological symptoms).

Histology

Histological studies confirmed the presence of AR in all patients clinically suspect, and the absence of characteristic features of rejection in the control group. The mean number of portal tracts was 10.83 (range 4–19), and in all cases the samples were sufficient for diagnosis. In two cases the rejection was mild, in eight, moderate and in two, severe. The median value of endothelitis was 1.5, of ductal damage, 1.91 and of portal inflammation, 2.18. The mean value of RAI (acute rejection index) was 5.5 (3.5 in the mild rejections, 5.65 in the moderate rejections and 7 in the severe episodes).

Immunohistochemistry

The comparative data in immunohistochemical scoring among different liver biopsy groups are shown in Table 3. We found a marked expression of ICAM-1 in sinusoids from patients with AR. Additionally, perivenular and periportal hepatocytes showed ICAM-1 reactivity. In contrast, ICAM-1 sinusoid expression was significantly lower in control patients and no hepatocyte reactivity was found. After corticoid treatment, the ICAM-1 tissue expression decreased, and this difference was statistically significant in sinusoids and perivenular hepatocytes.

When we compared the results of cortico-sensitive and cortico-resistant AR episodes, we found that after treatment, no ICAM-1 hepatocyte expression was found in patients with cortico-sensitive AR. However, hepatocytes showed the same or increased reactivity than before treatment in cortico-resistant AR.

Discussion

Cell-to-graft adhesion mechanisms are central in the development of AR, which requires infiltration of immu-

nocompetent cells into the graft [13]. In these processes, adhesion molecules play an important role. These molecules have been divided in three major families: selectins, integrins and immunoglobulin superfamily [14, 15]. ICAM-1 is a cell surface glycoprotein of 90 kDa and is a member of the immunoglobulin superfamily. ICAM-1 is expressed on a few cell types, but some inflammatory mediators, including interferon-γ, interleukin-1 and TNF-α, cause strong induction of this cellular adhesion molecule [16]. In the normal liver, ICAM-1 is expressed weakly on sinusoidal endothelia and some kupffer cells [17]. During AR episodes, the expression of ICAM-1 on hepatocytes has been demonstrated in liver biopsies [9, 12, 18, 19]. In the present study, we showed that during AR episodes, sinusoid ICAM-1 expression increased significantly and hepatocytes showed ICAM-1 reactivity, which was negative in the control group. Additionally, the increase in ICAM-1 expression during AR has been demonstrated in bile [20] and serum [11, 20], although the utility of serum ICAM-1 levels in monitoring the occurrence of rejection is controversial. Navarro [20] did not find that ICAM-1 elevation correlated with rejection. In contrast, Ninova [11] has found that ICAM-1 serum levels are increased during AR episodes, but are unchanged in other graft dysfunctions such as CMV hepatitis.

After treatment with an intravenous bolus of glucocorticoids, ICAM-1 tissue reactivity decreased significantly in sinusoids and perivenular hepatocytes. In periportal hepatocytes, the ICAM-1 tissue expression also decreased, although this difference did not reach statistical significance. Remarkably, our study showed that there was no ICAM-1 hepatocyte expression in resolved AR episodes. In contrast in the two patients with cortico-resistant AR the ICAM-1 hepatocyte reactivity did not change or increase. These observations may confirm the role of ICAM-1 in inflammatory processes. When rejection persisted with all the associated inflammatory components, ICAM-1 expression was not modulated by glucocorticoids.

In recent years, several authors [21–23] have suggested that immunotherapy directed at ICAM-1 may be efficacious in inhibiting host immune responses to hepatocytes and prolonging allograft survival. Best results seem to be obtained with a 3-day course of treatment rather than one-shot injection of monoclonal anti-ICAM-1 antibodies [24]. Manipulation of the adhesion molecule interaction by monoclonal antibodies may be a new mode of immunosuppresive treatment in liver transplantation.

In summary, this study confirmed the induction of ICAM-1 expression on hepatocytes during episodes of AR. Treatment with high doses of intravenous glucocorticoids induced a down-regulation in ICAM-1 tissue expression when the AR was cortico-sensitive. In contrast, no modulation was observed in cortico-resistant episodes.

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