Available online at www.sciencedirect.com

SCIENCE DIRECT

Transplant Immunology 15 (2005) 165 – 172

Transplant Immunology

www.elsevier.com/locate/trim

Differential expression of tissue factor (TF) in calcineurin inhibitor-induced nephrotoxicity and rejection—implications for development of a possible diagnostic marker

C. Österholm [a], B. Veress [b], M. Simanaitis [b], U. Hedner [c], H. Ekberg [a,*]

[a] Department of Nephrology and Transplantation, Lund University, Malmö University Hospital, S-205 02 Malmö, Sweden
[b] Department of Pathology, Lund University, Malmö University Hospital, Malmö, Sweden
[c] Research and Development, Novo Nordisk A/S, Måløv, Denmark

Received 4 May 2005; received in revised form 30 May 2005; accepted 6 June 2005

Abstract

Deposition of fibrin in the form of fibrinoid necrosis is a common feature of severe acute renal allograft rejection. The role of the coagulation system and its initiator tissue factor (TF) during this process is, however, still poorly understood. In this study, we analyzed the expression of TF in 88 renal transplants afflicted with different forms of rejection and calcineurin inhibitor-induced nephrotoxicity, to see whether there was differential expression of this protein. TF immunoreactivity was evaluated semiquantitatively in six different renal structures: the podocytes, Bowman epithelium, the endothelium of the glomeruli, the brush border of tubular cells, the thin ascending loop of Henle, and small arteries/arterioles. The TF expression of normal renal tissue ($n=6$) was restricted to the glomerular podocytes and Bowman epithelium, and to some extent the ascending loop of Henle. Renal allografts undergoing acute rejection (AR) of grades I–III, ($n=13$, $n=17$ and $n=12$, respectively) did not show any altered TF expression in the glomeruli or vascular endothelium. In the ascending loop of Henle, a reduced expression could be seen (ARI, $p=0.015$; and ARII, $p=0.043$). TF staining of the brush border of renal transplants undergoing acute cyclosporin A (CsA) nephrotoxicity ($n=18$) was significantly higher than in normal kidneys ($p=0.0003$), as well as in transplants undergoing various degrees of acute rejection (ARI, $p=0.027$; ARII, $p=0.0012$; and ARIII, $p=0.0001$). Tubular brush border-expressed TF was also evident in 10 of 15 allografts suffering from chronic CsA nephrotoxicity, compared to 4 out of 13 cases with chronic allograft vasculopathy (CAV), but the increase was not statistically significant relative to normal kidneys. The majority of the grafts afflicted with either of the two chronic conditions displayed a TF-positive arterial endothelium (CAV, $p=0.0034$; and chronic CsA nephrotoxicity, $p=0.0026$) relative to controls. In conclusion, these results indicate that vascular TF expression is not altered during acute rejection, but may be of importance in chronic allograft nephropathy. Furthermore, TF immunoreactivity in the tubular brush border may be specific to acute CsA nephrotoxicity and might be used as a biomarker for this condition. Further studies are required to evaluate the possible role of brush border-expressed TF in the pathogenesis of CsA nephrotoxicity.

Keywords: Tissue factor; Calcineurin inhibitor; Nephrotoxicity; Acute rejection; Chronic rejection

1. Introduction

Calcineurin inhibitors effectively prevent acute rejection and increase short-term renal allograft survival. However, long-term graft survival is not improved to the same extent and chronic allograft nephropathy (CAN) has emerged as a leading cause of graft loss, in addition to death with functioning graft. Various factors, such as prolonged cold ischemia time and reperfusion injury, and also viral infection, are believed to contribute to the process of CAN [1,2]. However, a recent study by Nankivell et al. has shown explicitly that the vast majority of renal grafts afflicted with CAN display lesions that may be associated with drug-induced nephrotoxicity, as a result of treatment with cyclosporin A (CsA) and tacrolimus [3,4].

* Corresponding author. Tel.: +46 40 33 37 41; fax: +46 40 33 73 35.
E-mail address: henrik.ekberg@kir.mas.lu.se (H. Ekberg).

0966-3274/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.trim.2005.06.001

Acute calcineurin inhibitor-induced nephrotoxicity (hereafter referred to as CsA nephrotoxicity) manifests clinically as elevated serum creatinine levels and decreased glomerular filtration rate (GFR), and can be reversed with appropriate management, i.e. dose reduction, or withdrawal of CsA, or by switching to a non-nephrotoxic drug. Chronic CsA nephrotoxicity, on the other hand, is an irreversible condition. The diagnosis mainly relies on histological findings in biopsies, such as hyalinization of arterioli, striped interstitial fibrosis and tubular microcalcification [3–5]. Although these findings are considered to be characteristic histological markers, the discrimination between chronic allograft vasculopathy (CAV) and chronic CsA nephrotoxicity remains a challenge, as the two conditions show certain clinical and histological similarities.

Changes associated with acute rejection are classified into three different grades (Banff grades I–III) according to the severity of mononuclear infiltration of the interstitium and tubuli [5]. Chronic allograft vasculopathy (CAV), also referred to as true chronic rejection in the Banff classification, is characterized by progressive intimal hyperplasia of the arteries/arterioli and interstitial fibrosis [5].

Tissue factor (TF), the physiological initiator of blood coagulation, is normally present in the peripheral structures of organs and this distribution provides a hemostatic barrier in traumatic injury [6]. It is abundantly expressed in the vascular adventitia of blood vessels and moderately expressed in the media. Endothelial cells facing the lumen are totally devoid of TF. In the kidney, TF expression is restricted to the glomerular podocytes, Bowman epithelium and, to some extent, the tubular loop of Henle. Apart from its role in initiation of hemostasis, TF has also been identified as a mediator of arteriosclerosis, tumor metastasis and angiogenesis [7–9]. Several studies have also described an effect of coagulation factors (including TF) that increases inflammation as well as fibrosis [10,11].

Deposited fibrin (in the form of fibrinoid necrosis) is the end-product of the blood coagulation cascade and is characteristic of severe acute rejection of renal allografts; yet, the role of the coagulation cascade during this process is poorly understood. In the healthy body, the natural anticoagulants predominate and strictly control the activities of the pro-coagulants, thus maintaining a non-thrombogenic environment. However, the presence of inflammatory mediators, such as the cytokines TNF-α, IL-1β and IL-6, has a propensity to shift the hemostatic balance in favor of coagulation and thrombosis (reviewed in [12]).

2. Objective

The objectives of this study were as follows. Firstly, we wanted to determine whether the intensity and distribution pattern of TF expression in renal allografts could be correlated to the presence and the severity of acute rejection and acute CsA nephrotoxicity. Secondly, we aimed to determine whether the distribution of TF was different in allografts showing histological signs of CAV to grafts afflicted with chronic CsA nephrotoxicity.

3. Materials and methods

3.1. Tissues

Biopsy material and surgical specimens were obtained from the Department of Pathology at Malmo University Hospital. Twenty biopsies, previously acquired by ultrasound-guided needle sampling, were selected randomly from each of six groups of diagnoses from the clinical database in addition to a control group. The conditions had previously been clinically suspected and histologically confirmed according to the Banff criteria [5]. The six groups were: groups 1–3, acute rejection (AR I–III); group 4, CAV; group 5, acute CsA nephrotoxicity; and group 6, chronic CsA nephrotoxicity. In group 5, trough concentrations of CsA were

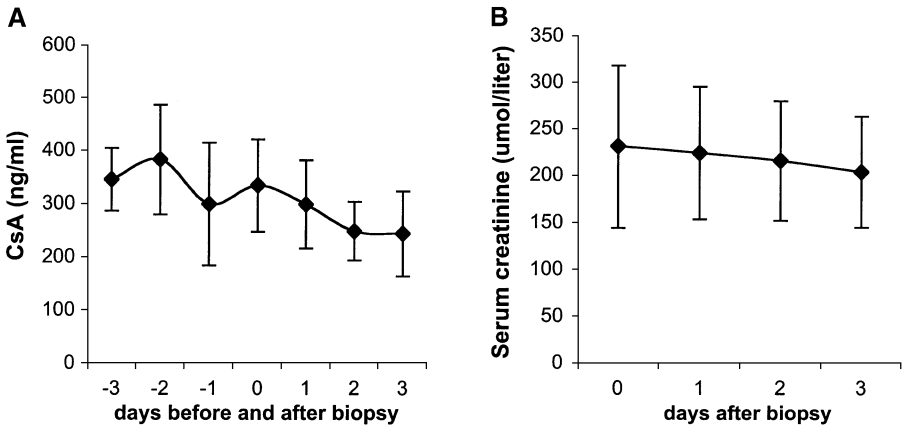

Fig. 1. (A) Trough concentrations of CsA (ng/ml) on the days before and after biopsy. Levels were high but declined upon dose reduction. (B) Levels of serum creatinine (μmol/l) slowly decreased on the days following biopsy retrieval. The biopsies showed no histological evidence of acute rejection, but instead changes due to drug nephrotoxicity.