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CD16$^+$ monocytes with smooth muscle cell characteristics are reduced in human renal chronic transplant dysfunction

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ABSTRACT

In chronic transplant dysfunction (CTD), persistent (allo)immune-mediated inflammation eventually leads to tissue remodeling including neointima formation in intragraft arteries. We previously showed that recipient-derived neointimal α-SMA$^+$ smooth muscle-like cells are present in human renal allografts with CTD. Human PBMC contain myeloid cells capable of differentiating into α-SMA$^+$ cells *in vitro*; the phenotype of the ancestral subset is as yet unknown. This study aimed to investigate whether monocyte subsets contain cells with smooth muscle-like cell differentiation capacity and whether CTD in renal transplant recipients is associated with a shift in these monocyte subsets. To accomplish this goal, monocyte subsets from healthy controls were sorted based on CD14 and CD16 expression to investigate gene expression levels of mesenchymal markers α-SMA and SM22α. CD14$^+$/CD16^{++} monocytes displayed increased α-SMA and SM22α mRNA expression compared with CD14^{++}/CD16$^-$ monocytes, suggesting increased differentiation potential toward smooth muscle-like cells. Flow cytometry revealed that in non-CTD transplant recipients the percentage of CD14$^+$/CD16^{++} monocytes was reduced, with an even further reduction in patients with CTD. To determine a potential correlation between CD14$^+$/CD16^{++} monocytes and α-SMA$^+$ cell outgrowth potential *in vitro*, PBMC of healthy controls and transplant recipients with and without CTD were cultured under fibrotic culture conditions, and indeed a significant correlation ($p = 0.0002$, $r = 0.62$) was observed. Finally, double staining for α-SMA and CD16 revealed presence of α-SMA$^+$CD16$^+$ cells in kidney explants from CTD patients, albeit at very low numbers.

Our data represent evidence that, compared to CD14^{++}CD16$^-$ monocytes, CD14$^+$CD16^{++} monocytes have an increased expression of smooth muscle cell-associated genes. This monocyte subpopulation is reduced in renal transplant patients with CTD, possibly due to selective migration into the allograft.

Introduction

Chronic renal transplant dysfunction (CTD), clinically evidenced by a gradual decline in kidney function and proteinuria, is histologically characterized by chronic transplant glomerulopathy, interstitial fibrosis and tubular atrophy (IF/TA), and transplant vasculopathy (TV) (Chapman et al. 2005; El-Zoghby et al. 2009; Solez et al. 2007). TGF-β1 is recognized as a strong inducer of tubulointerstitial fibrosis in CKD (Lopez-Hernandez and Lopez-Novoa 2012), including renal CTD (Djamali and Samaniego 2009). Furthermore, TGF-β1 serum levels have been associated with development of end-stage renal disease (Suthanthiran et al. 1998), and renal transplant recipients with CTD were shown to have increased TGF-β1

Abbreviations: α-SMA, α-Smooth muscle actin; CTD, chronic transplant dysfunction; ECM, extracellular matrix; IF/TA, interstitial fibrosis and tubular atrophy; PBMC, peripheral blood mononuclear cells; SMC, smooth muscle cell; TGF-β1, transforming growth factor-β1.

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serum levels when compared with controls (Campistol et al. 1999). These data strongly suggest that TGF-β1 is involved in the pathogenesis of CTD.

TV is defined by the development of an occluding hyperplastic neointima, which consists of proliferating smooth muscle cells (SMCs) between the endothelial cells and the internal elastic lamina. Multiple underlying factors, including ischemic injury during transplantation, the alloresponse to the transplanted kidney as well as non-alloimmune factors such as elevated blood pressure and proteinuria contribute to the development of TV. SMCs in the neointima secrete extracellular matrix (ECM) and cause luminal narrowing of the artery. This will lead to stiffening of the arteries and reduced blood flow in the downstream vascular bed. We have previously shown that recipient-derived α-SMA$^+$ cells are present in the neointima in human renal transplantectomy samples with CTD (Boersema et al. 2009). These α-SMA$^+$ cells can participate in the progression of fibrotic processes. A subset of peripheral blood mononuclear cells (PBMC) can differentiate into α-SMA$^+$ cells *in vitro* (Simper et al. 2002), although the phenotype of circulating precursors of these cells is still unclear. It is likely that recipient-derived α-SMA$^+$ intimal cells originate from PBMC which start to express α-SMA after migration into the injured artery, where they contribute to TV. Previous *in vitro* studies showed that within the mononuclear cell pool, a cell population resides which has the ability to differentiate into α-SMA-expressing smooth muscle-like cells (Simper et al. 2002; Sugiyama et al. 2006). Based on their retained expression of CD68 and CD14, these cells are most likely of myeloid origin (Metharom et al. 2008).

In humans, peripheral monocytes form a heterogeneous cell population which can be divided into different subsets based on the expression of the lipopolysaccharide receptor CD14 and co-expression of the FcγIII receptor CD16. The majority (~85%) of monocytes express high levels of CD14 without CD16 co-expression (i.e. CD14^{++}/CD16$^-$ classical monocytes). In addition, a minor monocyte subset (~10%) expresses lower levels of CD14 but high levels of CD16 (i.e. CD14$^+$/CD16^{++} non-classical monocytes) (Grage-Griebenow et al. 2001). Transcriptional profiling of CD16$^+$ and CD16$^-$ circulating monocytes confirmed that these monocyte populations originate from a common myeloid precursor (Ancuta et al. 2009). However, a number of genes are differentially expressed and suggests different biological functions *in vivo* for CD16$^+$ and CD16$^-$ monocytes (Ancuta et al. 2009). Compared to the major subset of CD14^{++}/CD16$^-$ classical monocytes, the non-classical CD14$^+$/CD16^{++} have lower phagocytic activity and produce higher levels of TNF-α and IL-1β (Belge et al. 2002; Thieblemont et al. 1995). In addition to the classical and non-classical monocytes, a small population (~5%) of CD14^{++}/CD16$^+$ intermediate monocytes can be identified in the human peripheral blood (Ancuta et al. 2003; Ziegler-Heitbrock et al. 2010). Based on their immunological function, classical, intermediate and non-classical monocytes exhibit different features and present different biologic properties *in vivo* (Hristov and Weber 2011). As yet, it is unknown whether these various monocyte subsets also differ in expression of mesenchymal markers which would favor the enrichment of circulating smooth muscle precursor cells within these monocyte subpopulations. We postulate that a circulating precursor for smooth-muscle-like cells is enriched within a certain monocyte subpopulation. Identification of this monocyte subset, which may contribute to CTD after renal transplantation, can aid the development of new therapeutic strategies to attenuate the development of CTD, in particular TV. We therefore tested the association between the frequency of monocyte subsets and PBMC α-SMA$^+$ cell outgrowth potential in healthy control subjects and renal transplant recipients with or without CTD.

Materials and methods

Subjects

In this cross-sectional study, 13 renal transplant patients with CTD were recruited from the renal transplantation outpatient clinic of the University Medical Center Groningen. Relevant transplant characteristics such as age, gender, and date of transplantation were extracted from the Groningen Renal Transplant Database which contains information on all renal transplantations performed at our center since 1968 (Zelle et al. 2011). Current medication was taken from the medical record. Inclusion criteria for CTD were urine protein excretion of >1.0 g/24 h, accompanied by increasing plasma creatinine concentrations as measured during the previous visit to the outpatient clinic. For comparison, matched renal transplant recipients without CTD (i.e. protein excretion <1.0 g/24 h, stable renal function; $n = 14$) were recruited. Matching was performed based on age, gender and time since transplantation. Healthy control subjects ($n = 12$) were matched for age and gender. Study participant characteristics are shown in Table 1. As anticipated, patients with CTD had higher serum creatinine and blood urea levels as well as proteinuria compared with non-CTD patients. Patients maintained their regular medication. No differences were present with regard to the immunosuppressive regimen between renal transplant recipients with or without CTD (Table 2). The number of WBCs/ml blood was determined using a Coulter

Table 1
Characteristics of healthy controls and renal transplant recipients with and without chronic transplant dysfunction (CTD).

	HC $N = 12$	Non CTD $N = 14$	CTD $N = 13$	p-Values
Donor characteristics				
Age, years (mean ± SD)		45.3 ± 17.6	42.2 ± 17.2	0.731
Female (%)		9 (69)	3 (25)	0.027
Transplant characteristics				
Cold ischemia time (h)		12 ± 10	14 ± 11	0.710
1st warm ischemia time (min)		5 ± 9	4 ± 7	0.701
2nd warm ischemia time (min)		39 ± 10	36 ± 9	0.361
No. of HLA mismatches		3 ± 1	2 ± 1	0.777
Characteristics of included subjects				
Age, years (mean ± SD)	43.6 ± 11.4	50.1 ± 11.4	46.5 ± 13.0	0.400
Female (%)	4 (33)	4 (29)	4 (31)	0.966
Time since TX, years (mean ± SD)		6.1 ± 4.6	7.1 ± 7.1	0.686
Creatinine (μmol/L)		141 ± 35	199 ± 92	0.043
Urea (mmol/L)		10.2 ± 3.2	16.9 ± 9.3	0.023
Proteinuria (g/24 h)		0.3 ± 0.4	2.5 ± 2.5	0.009
Mean blood pressure (mmHg) (mean ± SD)		112 ± 10	112 ± 14	0.960