Clinical Biochemistry 46 (2013) 1383–1388

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

CD26/dipeptidyl peptidase IV: A comparative study of healthy persons and kidney transplant recipients before and early after transplantation

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ARTICLE INFO

Article history:
Received 15 November 2012
Received in revised form 2 April 2013
Accepted 3 April 2013
Available online 19 April 2013

Keywords:
T cell activation
Biomarkers
Kidney transplantation
CD26
Dipeptidyl peptidase IV activity
sCD26

ABSTRACT

Objectives: Human CD26 is co-stimulatory for lymphocytes, circulates in a soluble form in blood (sCD26), and has intrinsic dipeptidyl peptidase IV (DPPIV) activity. Associations between CD26 expression on the surface of T cells (CD26+/CD3+) and acute rejection and between (CD26+/CD3+)/DPPIV and clinical immunosuppression have been reported. These results encouraged the investigation of CD26 as a potential biomarker to optimize immunosuppressive therapy. To better understand the significance of CD26, a comparative study of CD26 expression on CD3+ cells, sCD26 concentration, and DPPIV activity in healthy persons (HP) and kidney transplant recipients (KTR) was performed.

Design and methods: Thirty-one HP and 34 KTR were included in the study. CD26+/CD3+ was determined by FACS, sCD26 concentration was determined by ELISA, and DPP activity was determined by spectrophotometry. For KTR, these parameters were studied on the day before transplantation (preTx) and 7 ± 1 days after transplantation (postTx).

Results: There was no significant difference in the CD26+/CD3+, sCD26, and DPPIV data regarding gender, donor type (16 living donors), delayed graft function (n = 8), or presence of ≥4HLA mismatches (n = 16). Compared to the HP data, preTx CD26+/CD3+ was 4.5-fold higher, sCD26 was 1.2-fold higher, and DPPIV showed no significant difference. PostTx, CD26+/CD3+ was 3.8-fold higher, and sCD26 and DPPIV decreased significantly, reaching lower values than that observed in HP. Re-transplanted patients (n = 5) showed significantly lower preTx CD26+/CD3+ expression than patients receiving their first transplant. Patients with preemptive transplantation (n = 7) showed higher postTx CD26+/CD3+ expression than patients on dialysis.

Conclusions: CD26 expression on CD3+ cells was strongly increased in patients with end stage kidney disease compared to HP and remained high early postTx. The differences in sCD26 and DPPIV behavior compared to that of CD26+/CD3+ postTx may reflect a regulatory response to the new immunological situation and the effects of therapy.

Introduction

T cell activation antigen CD26, also known as dipeptidylpeptidase IV (DPPIV), is a transmembrane glycoprotein and a member of the S9B serine peptidase protein family [1]. It is expressed in a dimeric form in a variety of immune (e.g., mature thymocytes, activated T cells, B cells, NK cells, and macrophages) and non-immune (e.g., renal, prostate, liver and small intestinal epithelium, biliary canaliculae, and splenic sinus lining cells) cells [2]. Hopsu-Havu and Glenner first described the DPPIV activity (dipeptide naphthylamidase hydrolyzing glycyl-prolyl-beta-naphthylamide) of this protein in the human liver approximately 45 years ago [3]. Two years later, Nagatsu et al. discovered the serum DPPIV activity [4], and in 1984, Fox et al. identified the protein as a leukocyte antigen [5]. Additionally, the occurrence of significant DPPIV activity has been reported for cerebrospinal and synovial fluid, semen and urine [5]. Although it is accepted that the predominant (~90%) DPPIV activity measured in blood originates from soluble CD26 protein (sCD26) shed from the membrane of CD26-expressing cells, there are some other circulating proteins that possess DPPIV activity (e.g., other DPPs). Moreover, there is evidence suggesting that DPPIV activity in serum is regulated by the degree of sialylation of the protein or the interaction of this protein with attractin [6]. Taken together, these features can result in the absence of a direct correlation between sCD26 concentration and enzyme activity.

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http://dx.doi.org/10.1016/j.clinbiochem.2013.04.006

CD26/DPPIV is a multifunctional molecule involved in a variety of biological processes. However, a particularly important aspect for clinical transplantation is the role of CD26/DPPIV in immune regulation. Multiple studies in recent years have demonstrated the link between this molecule and signaling pathways and structures involved in T cell activation, as well as antigen-presenting cell-T cell interaction (co-stimulation), cytokine secretion (e.g., IL-2, IL-17, and IL16), and immunoglobulin production, as reviewed in [1,6–8]. Some immunoregulative hormones and chemokines have been reported to be substrates of CD26/DPPIV (e.g., neuropeptide Y, CCL3 and 4, RANTES, and CXCL9-12). This protein has been shown to be a receptor for adenosine deaminase, to interact with proteins of the extracellular matrix (collagen and fibronectin), and to mediate signaling by direct interaction with CD45, glypican-3, chemokine receptor CXCR4, mannose 6-phosphate/insulin-like growth factor II (M6P/IGF IIR), and, most recently, caveolin-1 [1,6–8]. Inhibition of DPPIV activity by synthetic inhibitors leads to the suppression of T cell proliferation in vitro, a decrease in antibody production in vivo [1,9], downregulation of the production of inflammatory cytokines, and upregulation of anti-inflammatory cytokines [10].

Despite increasing amounts of knowledge regarding the physiological role of CD26/DPPIV, there is currently no conclusive information about the behavior of antigen expression, sCD26 concentration, and enzymatic function under disease conditions, particularly in transplantation. As described by Cordero et al. [6], CD26/DPPIV effects can be dependent on or independent of enzymatic activity. Moreover, recent experimental results by Ansorge et al. [10] show that the molecular mechanism of action of the membrane-bound enzyme may differ from that of the soluble form, and the membrane-bound form may be preferentially involved in processes related to the immune response. The results from some individual studies demonstrated that CD26/DPPIV may be a promising biomarker for monitoring the immune status in solid organ transplantation. Korom et al. [11] reported a decreased serum DPPIV activity during clinical immunosuppression in transplant recipients. In a rat model of allogenic heart transplantation, DPPIV activity was increased preceding rejection, and the presence of a synthetic DPPIV inhibitor ameliorated the rejection [12]. In addition, in a rat lung transplantation model, DPPIV inhibition has been shown to attenuate post-transplantation ischemia/reperfusion injury and preserve early graft function after extended ischemia [13]. Our group recently presented data on an association between low CD26 expression on the surface of T cells and freedom of rejection during the first 3 months after kidney transplantation. Included in the study were 35 kidney transplant recipients treated with basiliximab, tacrolimus, mycophenolate sodium, and steroids [14]. Moreover, direct dose- and time-dependent inhibitory effects of tacrolimus, sirolimus, everolimus, cyclosporine A and mycophenolic acid on the CD26 expression on CD3+ cells in vitro were demonstrated [15].

Monitoring CD26/DPPIV is attractive from an analytical point of view. The membrane-bound fraction can be easily measured by flow-cytometry directly in whole blood without time-consuming leukocyte isolation, ex-vivo incubation and stimulation required for studying other T cell activation markers, such as CD25 or CD71. Enzyme activity can be followed by a simple chromogenic or fluorogenic assay, and ELISA kits are commercially available for sCD26 determination. Whereas CD26 expression on lymphocytes is stable up to 3 days in whole blood, samples for sCD26 and enzyme activity measurements can be stored frozen over several months and are appropriate for multicenter clinical studies [16]. Because CD26 expression is preferentially found on Th1 cells known to be involved in the inflammatory response accompanying graft rejection, this marker can provide information in addition to CD30 (and particularly sCD30), a predominantly Th2 associated protein, for which a role as a general biomarker has been already elucidated in some large clinical transplantation studies [6,16].

Given this background, we were encouraged to initiate this single-center study aimed at generating data on the relationship between the 3 manifestations of the protein i.e. CD26 expression on T cells, sCD26 concentration, and DPPIV activity in kidney transplant patients. Investigating these relationships prior to and shortly after transplantation, as well as in healthy persons, will enable a better understanding of CD26/DPPIV as a potential biomarker in clinical transplantation.

Materials and methods

Recruiting of the control group

Healthy blood donors (HP, n = 31, 16 males and 15 females) were recruited during a blood donation appointment. The age range was 23 to 69 years, with a median age of 44. Each blood donor gave an additional 2.6 mL EDTA plasma during his or her blood donation. Cytometric data were retrieved within a few hours after the blood donation. Plasma for the investigation of DPPIV activity and sCD26 levels was frozen at −20 °C and analyzed together in one batch. None of the blood donors had been sick during the four weeks before blood donation or had been taking any medication, including oral contraceptive pills.

Recruiting of the patient group

Kidney transplant recipients (KTR, n = 34) were recruited during the transplantation process. The median age was 48, and the age ranged from 26 to 76 years. Of the 34 KTR, 20 were male and 28 were female. While 18 of the 34 KTR received an organ from a deceased donor, 16 KTR received an organ from a living donor. For 29 patients, it was their first transplant, while 5 KTR were receiving a second transplant. Preemptive transplantation was possible in 7 KTR, while 27 patients received dialysis (hemodialysis or peritoneal dialysis) prior to transplantation. HLA typing of kidney recipients and donors showed that 15 KTR received a kidney with four or more HLA mismatches (HLA-A, -B, and -DR), while 19 KTR received an organ with fewer than 4 HLA mismatches.

Blood collection in patients

The first blood sampling was performed prior to transplantation (preTx), either one day before or a few hours before transplantation. On day 7 (±1) after transplantation (postTx), another blood sample was collected from the KTR. The blood sampling time point at day 7 (±1) after transplantation was chosen in this study because it was found to best recognize the risk of early acute rejection in a preliminary study of our group with kidney transplant recipients (14). After establishing the cytometric results, the plasma preTx and postTx was frozen at −20 °C and used for the determination of DPPIV activity and sCD26 protein concentration, which were performed in batches.

Standard medication

Patients received 250 mg i.v. of prednisolone (solu-Decortin H®) 4 h before transplantation and another 250 mg 6 h after transplantation. On the first post-operative day, 50 mg of prednisolone was administered i.v., and given orally from the second post-operative day forward. The dosage was initially 0.5 mg/kg body weight and was reduced to 20 mg/day at day 15. Another reduction to 15 mg/day occurred two weeks later. Finally, prednisolone was reduced by 2.5 mg every two weeks, until the dosage of 5 mg/day was received 3 months after transplantation.

KTR received 20 mg i.v. basiliximab (Simulect®) 4 h before transplantation and another 20 mg i.v. on day 4 after transplantation.

Initially, the patients were given 0.10 mg/kg body weight tacrolimus (Prograf®) twice daily. Then, the dosage was adjusted to a trough concentration of 6–8 μg/L during the first 3 months after transplantation.