Transplant Immunology 23 (2010) 161–165

Contents lists available at ScienceDirect

Transplant Immunology

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

Soluble CD30 and Cd27 levels in patients undergoing HLA antibody-incompatible renal transplantation

Rizwan Hamer [a,d,*], Laura Roche [b], David Smillie [b], Andrea Harmer [b], Daniel Mitchell [d], Guerman Molostvov [d], For T. Lam [a], Habib Kashi [a], Lam Chin Tan [a], Chris Imray [a], Simon Fletcher [a], David Briggs [c], David Lowe [c,d], Daniel Zehnder [a,d], Rob Higgins [a]

[a] Transplant Unit, University Hospitals Coventry and Warwickshire, Coventry, West Midlands, UK
[b] H&I Laboratory, National Blood Service, Sheffield, UK
[c] Histocompatibility Laboratory, National Blood Service, Birmingham, West Midlands, UK
[d] Clinical Sciences Research Institute, Warwick Medical School, University of Warwick, Coventry, West Midlands, UK

ARTICLE INFO

Article history:
Received 27 March 2010
Received in revised form 2 June 2010
Accepted 10 June 2010

Keywords:
CD30
CD27
HLA antibody-incompatible transplantation

ABSTRACT

HLA antibody-incompatible transplantation has a higher risk of rejection when compared to standard renal transplantation. Soluble CD30 (sCD30) has been shown in many, but not all, studies to be a biomarker for risk of rejection in standard renal transplant recipients. We sought to define the value of sCD30 and soluble CD27 (sCD27) in patients receiving HLA antibody-incompatible transplants. Serum taken at different time points from 32 HLA antibody-incompatible transplant recipients was retrospectively assessed for sCD30 and sCD27 levels by enzyme-linked immunosorbent assay (ELISA). This was compared to episodes of acute rejection, post-transplant donor-specific antibody (DSA) levels and 12 month serum creatinine levels. No association was found between sCD27 and sCD30 levels and risk of acute rejection or DSA levels. Higher sCD30 levels at 4–6 weeks post-transplantation were associated with a higher serum creatinine at 12 months.
Conclusion patients undergoing HLA antibody-incompatible transplantation are at a high risk of rejection but neither sCD30 (unlike in standard transplantation) nor sCD27 was found to be a risk factor. High sCD30 levels measured at 4–6 weeks post-transplantation was associated with poorer graft function at one year.

1. Introduction

The incidence of chronic kidney disease (CKD) is increasing globally and consequently the need for renal transplantation is on the rise [1,2]. To match this need renal transplantation previously considered impossible, such as HLA antibody-incompatible transplantation (AIT), have been increasingly attempted [3]. Despite recent successes the risk of rejection in these patients remains high [4]. Various studies have attempted to look for markers to identify individuals at higher risk of rejection with the aim of tailoring immunosuppression [5]. One marker that has recently shown promise as an indicator for acute rejection and decreased graft survival is soluble CD30 [6,7].

CD30 is a 120 kD membrane glycoprotein molecule belonging to the tumor necrosis factor (TNF) receptor superfamily. It was first discovered as a surface antigen on the Hodgkin Reed Sternberg cell [8] and has since been found to be expressed on human CD4+ and CD8+ Th2 cells, natural killer cells and a few non-lymphoid cells [9]. Activation of CD30+ cells results in cleavage of CD30 with the release of a soluble form (sCD30) that is detected at low levels in normal individuals. Pelzl et al. were the first to report a relationship between high pre-transplant sCD30 levels and graft rejection [6]. Subsequently, several reports have shown high pre- and post-transplant sCD30 levels to be associated with an increased risk of both acute and chronic graft rejection and reduced graft survival [7,10–13]. High pre-transplant sCD30 levels has not, however, always been shown to be associated with a higher risk for rejection [11]. The association between sCD30 levels and risk of AMR is less well understood although some groups have suggested a correlation between the two [14,15]. Slavcev et al. demonstrated higher sCD30 levels in patients with AMR and a lower risk for development of AMR and de novo donor-specific antibodies in patients with lower sCD30 levels [16].

CD27 is a 55 kD transmembrane disulphide-linked dimer which also belongs to the TNF receptor superfamily. It is expressed on peripheral blood T cells and a subset of B lymphocytes. Studies in the 1990s demonstrated that stimulation of CD27+ B cells resulted in a 5- to 100-fold more immunoglobulin synthesis when compared to stimulated CD27− B cells [17,18]. It was subsequently shown that peripheral blood and splenic CD27+ B cells had somatically mutated Ig V region genes whilst CD27− B cells were unmutated [19]. This led to the conclusion that CD27 expression is characteristic of human memory B cells. Interaction of CD27 on peripheral blood B cells with

* Corresponding author. University Hospitals Coventry & Warwickshire NHS Trust, Coventry, UK CV2 2DX. Tel.: +44 2476 964000.
E-mail address: rizwanhamer@yahoo.co.uk (R. Hamer).

0966-3274/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.trim.2010.06.004

the CD27 ligand (CD70) in the presence of IL-10 results in the differentiation of these B cells into plasma cells. CD27- cells do not differentiate into plasma cells in the presence of IL-10 and CD 70 transfectants [20]. High levels of soluble CD27 (sCD27) have been found in patients with autoimmune conditions involving T cell hyperactivity such as SLE [21] and rheumatoid arthritis [22] and in patients with B cell malignancies [23] and AIDS associated lymphoma [24]. Raised serum sCD27 has also been found to correlate with renal dysfunction [25].

All HLA AIT treatment protocols aim to reduce the donor-specific antibody (DSA) load prior to transplantation. Despite this the risk of antibody mediated rejection (AMR) of the graft is significant [26] and is often accompanied by a rise in HLA antibody titres following surgery. However, not all patients with AMR have a concomitant rise in antibody levels and antibody re-synthesis is not invariably associated with AMR. Identifying patients who are more likely to develop AMR has so far remained a challenge. We conducted a retrospective single-centre study to determine changes in sCD30 and sCD27 levels in patients undergoing HLA AIT, correlating these with DSA and episodes of rejection.

2. Materials and methods

HLA sensitised patients with DSA measured by complement dependant cytotoxic (CDC) crossmatch, flow cytometric (FC) crossmatch or microbead assay were enrolled into the AIT programme at University Hospitals Coventry and Warwickshire. All patients gave informed consent for enrolment into our study. Most patients underwent a course of double filtration plasmapheresis (DFPP) – usually 5 sessions – before transplantation with the aim of achieving a negative FC crossmatch. They were immunosuppressed with mycophenolate mofetil 1000 mg twice a day with dose reduction if white cell count fell below 4.0×10^9/l, tacrolimus 0.15 mg/kg/day in two divided doses aiming for a target trough level of 10–15 μg/l in the first month, prednisolone 20 mg once a day, intravenous 500 mg methylprednisolone intraoperatively and basiliximab 20 mg on days 0 and 4.

For the purpose of this study 32 patients had blood collected at various time points pre- and post-transplantation (Fig. 1). Blood samples were collected in BD Vacutainer blood collection tubes (containing clot activator for serum), immediately placed on ice and centrifuged at 3500 rpm for 15 min at 4 °C for plasma and serum separation. Samples were stored at −80 °C prior to processing. Repeat freeze–thaw cycles were avoided. Patient characteristics are shown in Table 1.

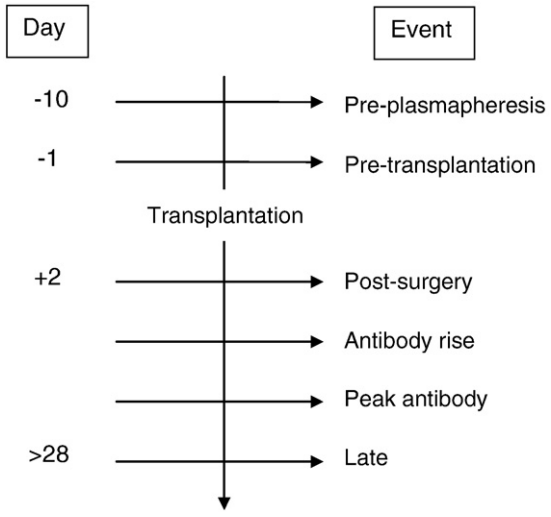

Fig. 1. Time points at which blood samples were collected.

Table 1
Patient characteristics (AMR = antibody mediated rejection).

		No AMR	AMR	*p* value
n		18	14	
Mean Age (range)		40(18–54)	41(23–60)	0.76
Female sex		44%	71%	0.14
ESRF (mean years ± SEM)		13.4 ± (2.27)	12.1(2.33)	0.68
Previous renal transplants	None	28%	36%	0.63
	One	61%	64%	0.86
	Two or more	11%	0%	0.2
CDC positive		28%	28.5%	0.97
Flow positive		56%	43%	0.48
Bead positive		16%	28.5%	0.39
Post-transplant dialysis		18%	28%	0.36
Death		5.5%	7%	0.86

Commercially available enzyme-linked immunosorbent assay (ELISA) kits were used to assess soluble serum CD30 and CD27 (Bender Medsystems GmbH) as per manufacturer's instructions.

Blood was also collected before and after each DFPP session prior to transplantation, and, following transplantation, daily for the first two weeks and three times a week for the next two weeks for antibody analysis. HLA antibody levels were measured with CDC crossmatching (not AHG enhanced), FC crossmatching, and microbeads (One Lambda Inc (Canoga Park, CA, USA), analysed on the Luminex platform (XMap 200)).

Patients were diagnosed to have an acute antibody mediated rejection episode on renal biopsy according to the Banff criteria [27]. A suspicious biopsy (7 cases) was considered to be positive for acute AMR if there was a concomitant decline in renal function and rise in DSA after other causes of renal dysfunction was ruled out. In one case rejection was diagnosed clinically in the setting of a rapid fall in urine output with a rise in both serum creatinine and DSA. Patients were classified as having severe rejection if they required dialysis in addition to enhanced immunosuppression for treatment of acute AMR.

Biopsies were fixed in 10% neutral buffered formalin and routinely processed into paraffin wax for standard light microscopy. Immunohistochemistry on de-waxed sections was undertaken using the following antibodies: C4d (Biomedica Gruppe C4dpAb, Cat No. BI-RC4D) and CD45 (DAKO CD45, Cat No. M0701 or Vision Biosystems CD45 X16/99, Cat No. PA0042).

Slides were reviewed and scored according to the Banff criteria for cellular (T-cell mediated) rejection, and by Banff 07 for PTC margination (grades PTC0–PTC3) and C4d staining (C4d0–C4d3). Additionally, the numbers of cells in all glomeruli in the sample and in at least 3 high power fields of cortical kidney were counted.

2.1. Statistics

Rejecter and non-rejecter parameters were compared using descriptive statistics. Comparisons between groups were performed using the Mann–Whitney *U* test and continuous variables compared using the Wilcoxon Signed Ranks test. Cox regression was used to determine any association between graft dysfunction and the measured variables. A p value of <0.05 considered to be significant. SPSS for windows, version 15, (SPSS Inc. Chicago IL) was used for all calculations.

3. Results

14/32 (44%) of patients developed an episode of antibody mediated rejection and 7/32 required dialysis in the first post-transplant week. 10/14 of the patients who developed rejection required treatment with OKT3 (muromonab) in addition to methylprednisolone. Rejection and non-rejection groups were similar with respect to gender, age at transplantation and number of years on dialysis (Table 1).