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Different regulatory and cytotoxic CD4$^+$ T lymphocyte profiles in renal transplants with antibody-mediated chronic rejection or long-term good graft function

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ABSTRACT

Comparative analysis of the different subsets of CD4$^+$ T-lymphocytes may provide hints on the immunologic mechanisms operating in the long-term fate of a kidney transplant.

We analyzed peripheral regulatory CD4$^+$ T cells (Tregs) and CD4$^+$ cytotoxic T lymphocytes (CTLs) in antibody-mediated chronic rejection (AMCR), in middle-term kidney transplants (2–4 years, MTKT) with good graft function and rejection-free history, in long-term kidney transplants (>15 years, LTKT) and in normal healthy subjects (NHS).

Transplant groups with good prognosis (MTKT and LTKT) displayed a significant lower amount of CD4$^+$CD25^{high} T lymphocytes than NHS, with a trend of a higher percentage in AMCR than in MTKT and LTKT. However, CD4$^+$CD25^{high} Foxp3$^+$ cells were significantly higher in LTKT and MTKT than AMCR. Characterization of CD4$^+$CD25^{high} T cells showed a marked increase of intracellular CTLA-4 in the AMCR group in respect to the other transplant groups, while the expression of the surface molecule seemed to follow a reverse trend. In addition, CD27, a costimulatory receptor involved in long-term T cell survival and prevention of immune tolerance, is significantly reduced in CD4$^+$CD25^{high} and CD4$^+$Foxp3$^+$ T cells in the LTKT in respect to the other transplant groups. CD4$^+$CD25^{high}CD45RO$^+$ and CD4$^+$Foxp3$^+$CD45RO$^+$ regulatory T cells with memory function were increased in LTKT compared to NHS and for the latter also in AMCR group. Finally, CD4$^+$CTLs that were quantified on the basis of granzyme A expression, were more represented in AMCR patients in comparison to the other groups. Strikingly, CD27 in the CD4$^+$CTLs was suppressed in LTKT and MTKT and markedly expressed in AMCR group. No significant differences in the expression of CD28 were observed among different groups.

In conclusion, different profiles of Tregs and CD4$^+$CTL populations correlate with different long-term conditions of kidney-transplanted patients, suggesting their role in the development of immunologic events in kidney transplantation.

1. Introduction

There is a consistent amount of data to support the potential involvement of specific populations of regulatory T cells in graft acceptance by the host immune system. In the last decade, a putative regulatory T cell population has been identified by the expression of CD4 and CD25, concomitantly to forkhead box P3 (FoxP3) transcription factor, and termed "Treg" [1–3]. The importance of FoxP3 expression for immune regulatory functions was suggested by the presence of severe autoimmune reactions in mice lacking its gene [4]. Consistently, human mutations of FoxP3 were shown to lead to the immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) [5].

Evidences of Treg involvement also in the immunologic phenomena occurring in solid organ transplantation, have been recently reported.

Abbreviations: Tregs, regulatory T cells; CTL, cytotoxic T lymphocytes; mAbs, monoclonal antibodies; GzmA, granzyme A; GzmB, granzyme B; Perf, perforin; NHS, normal healthy subjects; LTKT, long-term kidney transplants; MTKT, middle-term kidney transplants; AMCR, antibody-mediated chronic rejection; sCr, serum creatinine; FoxP3, forkhead box P3; CTLA-4, cytotoxic T-lymphocyte antigen 4; TNF, tumor-necrosis factor; GITR, glucocorticoid-induced TNF receptor family-related protein; IL-7, interleukin 7; MHC-II, major histocompatibility complex II; HLA, human leukocyte antigen; MMF, mycophenolate mophetil; CsA, cylosporine; Tac, tacrolimus; mTORi, mammalian target of rapamycin inhibitor; AZA, azathioprine; CIN, inhibitors of calcineurine; ST, steroid; ESRD, end stage renal disease; APKD, autosomal polycystic kidney disease; PRA, panel reactive antibodies; FITC, Fluorescein isothiocyanate; PE, phycoerythrin; TC, tri color (R-PE Cy5 Tandem); FCM, flow cytometry; PBS, phosphate-buffered saline; FACS, fluorescence activated cell sorting; APC, antigen presenting cell.

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Tregs' transfer in mice was shown to enhance graft acceptance in mice models of organ transplantation [6]. In the human setting, first evidence of antigen-specific Tregs in stable renal transplant recipients was provided by the study of Salama et al. [3] where it has been demonstrated that regulatory cells are present in the circulation of patients at different time-points after transplantation and persist for years, despite conventional immunosuppression. Moreover expression of FoxP3 mRNA in urine was associated with better recovery from rejection and improved long-term outcome [7].

Further characterization of Tregs has been attempted by several authors. In this view, expression of the costimulatory molecule cytotoxic T-lymphocyte antigen 4 (CTLA-4) and the tumor-necrosis factor (TNF)-superfamily member GITR (glucocorticoid-induced TNF receptor family-related protein) was observed [8,9]. By the functional point of view, the IL-7 receptor (CD127) expression inversely correlated with FoxP3 and suppressive function of Tregs [10]. Moreover, the direct ex vivo expression of MHC-II in the context of $CD25^{high}$ was shown to identify a mature, functionally distinct regulatory T cell population involved in contact-dependent in vitro suppression [11]. In human kidney transplantation, the establishment of a link between Tregs and long-term hyporesponsiveness has been attempted with non univocal results. In the work of Ruggenenti et al. [12] an enhanced expression of Tregs was observed under a low-dose sirolimus combined to alemtuzumab induction and mycophenolate mophetil (MMF)-based steroid-free maintenance therapy. However, this event did not appreciably protect renal transplant recipients from chronic allograft injury and dysfunction. On the other hand, clinically tolerant kidney graft recipients displayed normal levels of $CD4^{+}CD25^{high}$ T cells and FoxP3 transcripts whereas chronic rejection was associated with their decrease [13]. This observation suggests that tolerance may be due to a maintained phenomenon of natural tolerance that is lacking in patients with chronic rejection [13–15].

The discordant data concerning Treg activity in the human setting of kidney transplantation may potentially result from the counteractivity of other $CD4^{+}$ lymphocyte populations which influence the balance of the immune response. The presence of cytotoxic activities on specific subsets of $CD4^{+}$ T cells has been observed two decades ago [16,17]. The elucidation of the mechanisms of lymphocyte cytotoxicity has further contributed to their isolation and characterization. Indeed, the lymphocyte cytotoxic capacity depends not only on the secretion of proapoptotic cytokines such as TNF-α, but also on a mechanism dependent from the granule exocytosis of perforin (Perf) and granzymes A and B (GzmA, GzmB) [18,19]. Perforin forms pores that facilitate the delivery of granzyme into the cytosol which in turn activate an apoptotic cascade to initiate target cell death [20]. Based on the expression of these molecules, a population of $CD4^{+}$ cytotoxic T lymphocytes ($CD4^{+}$CTL), that seems to represent a highly differentiated antigen-experienced T cells, has been identified [18].

Transcriptome characterization of these cells has shown important similarities between $CD4^{+}$ and $CD8^{+}$CTL cells [21]. Their cytotoxic activity seems to be exerted mostly through perforin-dependent mechanism rather than Fas-dependent pathway [22]. Expression of these cytolytic molecules was studied using real-time PCR in the biopsy tissue, peripheral blood leukocytes, and urinary cells in kidney-transplanted patients with acute rejection or acute tubular necrosis [23], resulting in the possibility of distinguishing these two conditions. However, specific evaluation of $CD4^{+}$CTL in kidney transplantation has not been so far reported.

2. Objective

In the present work, we analyzed the expression and characterization of Tregs and $CD4^{+}$CTL in the kidney transplantation setting in antibody-mediated chronic rejection (AMCR), rejection-free kidney transplants with good graft function after 2–4 years (middle-term kidney transplant, MTKT) and >15 years post-transplantation (long-term kidney transplant, LTKT).

3. Materials and methods

3.1. Patients

In this study we have recruited three groups of kidney transplanted patients of the Torino Renal Transplant Centre, as described in Table 1:

Table 1
Clinical description of the different kidney-transplanted groups.

Groups		LTKT	MTKT	AMCR
Average age at transplant time (years)		40.56 (21–56)	53.23 (32–67)	43 (18–69)
Average age at the time of study (years)		60.5 (45–76)	57.3 (36–69)	54.4 (30–72)
Sex (M:F)		25:5	8:5	7:2
Cause of ESRD		Unknown, 12; Gn, 6; APKD, 3; malformative, 4; nephroangiosclerosis, 2; diabetic nephropathy, 1; Alport, 1; chronic pyelonephritis, 1; renal cortical necrosis,1	Unknown, 4; Gn, 1; APKD, 3; malformative, 1; nephroangiosclerosis, 3; diabetic nephropathy, 1	Unknown, 6; Hyperoxaluria 1; Gn 1; malformative, 1
First transplant (%)		90	100	77.7
Patients with history of acute rejection (%)		50	0	55.9
sCr (mg/dl)		1.35 (0.7–2.5)	1.35 (0.8–1.6)	3.3 (2.3–4.7)
Immunosuppressive therapy at the time of study	CsA	7	0	0
	CsA-AZA	5	0	0
	CsA-MMF	0	2	1
	CsA-ST	14	1	2
	CsA-ST-MMF	0	0	0
	Tac	0	4	0
	Tac-MMF	0	3	0
	Tac-ST	0	1	2
	Tac-ST-AZA	0	0	0
	mTORi -ST	0	1	0
	ST-AZA	4	0	0
	Tac-ST-MMF	0	0	2
	CsA+AZA+ST	0	0	1
	mTORi+CsA+ST	0	0	1

Data are reported as median (min–max). Abbreviations: LTKT, long-term kidney transplants; MTKT, middle-term kidney transplants; AMCR, antibody-mediated chronic rejection; AZA, azathioprine, mTORi, mammalian target of rapamycin inhibitor; MMF, mycophenolate mophetil; Tac, tacrolimus; CsA, cyclosporine A; sCr, serum creatinine; ST, steroid; ESRD, end stage renal disease; APKD, autosomal polycystic kidney disease.