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ORIGINAL ARTICLE

CD28 downregulation on CD4$^+$ T cells is associated with age of kidney transplant recipient

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Summary

There is a growing body of evidence showing that the intensity of rejection is weaker in older kidney allograft recipients while chronic complications, but not rejection, are the main causes of graft loss. To investigate whether the age of the recipient is a factor affecting the expressions of the CD28, CTLA-4, and CD40L costimulatory molecules on CD4$^+$ T cells. Their expression levels were determined in 78 kidney transplant recipients aged 17–68 years. The expression was assessed on unstimulated and anti-CD3 antibody + IL-2-stimulated CD4$^+$ T cells. Median time after transplantation was 20 months and median serum creatinine was 1.5 mg/dl. Significant correlations between age and CD28 expression ($r = -0.4$, $P = 0.0004$) on CD4$^+$ T cells and between age and CTLA-4 expression after stimulation ($r = 0.34$, $P = 0.008$) were found. CD40L expression on CD4$^+$ T cells was not affected by recipient age. The decreased expression of CD28 and enhanced expression of CTLA-4 (after stimulation) associated with age may be helpful in transplant acceptance.

Introduction

Patient death with a functioning graft is the major cause of allograft loss in elderly recipients. The leading causes of death in elderly transplant recipients are cardiovascular events, infections, and malignancies [1–4]. Some series reported infection as accounting for even 50% of deaths in an older group compared with less than 15% in younger patients [1,5]. Canadian and European registry data clearly showed that the risk of graft loss by rejection decreases with age [6,7]. This phenomenon may be explained by a gradual decline in responsiveness to antigens due to the emergence of immunosenescence.

The aging immune system exhibits diminished immune function in a variety of ways. For example, the main functions of T cells, i.e. activation, anergy, and apoptosis, are affected during aging. A variety of studies have documented that the proportion of T cells, both CD8$^+$ and CD4$^+$, lacking CD28 expression is dramatically increased in elderly immunocompetent individuals [8–11]. CD28− T cells have features of senescence, including oligoclonal expansion, shortened telomers, limited proliferation and decreased production of TNF-alpha and IL-6 [8,12]. The outcome of the antigen recognition and signal transduction induced by TCR engagement is dependent on effective costimulation. CD28 ligation is the major costimulatory signal for the activation of naive T cells. Its interaction with the natural ligand B7 family members CD80/86 expressed on antigen-presenting cells (APCs) results in costimulation and full activation of the T cell [13,14]. The termination of ongoing responses and the proliferation of activated CD4$^+$ T cells occur by the binding of cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) to B7, with a 20- to 100-fold higher affinity than CD28 [15]. A majority of CTLA-4 molecules are located in intracellular stores and CTLA-4 is only transiently expressed and rapidly endocytosed away from the cell surface following T-cell activation [15]. Some studies

also reported CTLA-4 upregulation during aging, which may additionally contribute to T-cell hyporesponsiveness [16,17].

The expression of the CD40L (CD 154) molecule represents one of the earliest and most specific T-lymphocyte activation markers during an immune reaction. Experimental data showed that the interaction of CD40L with CD40 on APCs directly transduces a positive signal to the cell's interior. It has also been found that part of the mechanism of action of the CD40/CD40L pathway is indirect, i.e. through the induction of CD28 ligands (B7 family) and the resultant activation of the CD28 pathway [18,19].

Diminished responsiveness to antigens in older individuals, which is documented in immunocompetent persons, may facilitate acceptance of a kidney allograft. Such recipients would benefit from a weaker immunosuppression regimen. On the other hand, immunosuppression might disturb alterations in T-cell functioning resulting from immunosenescence.

The aim of this study was to investigate whether recipient age is a factor modifying the expressions of CD28, CD40L and both surface (s) and intracellular (i) CTLA-4 on freshly drawn and anti-$CD3^+$rIL-2-stimulated peripheral blood $CD4^+$ T cells from kidney transplant recipients.

Material and methods

Patients

Seventy-eight kidney allograft recipients (34 females and 44 males) aged 17–68 years who underwent transplantation between January 1998 and March 2004 were studied after obtaining informed consent. The cause of end-stage renal disease was glomerulonephritis in 38 patients (48.7%), interstitial nephritis in eight (10.3%), polycystic kidney disease in seven (9%), diabetic nephropathy in five (6.4%), hypertensive nephropathy in four (5.1%), and other causes in 16 (20.5%). All patients received immunosuppressive regimens based on calcineurin inhibitor, azathioprine or mycophenolate mofetil and steroids. To exclude inflammatory reactions, the serum levels of C-reactive protein (hsCRP) were measured. Individuals with concentrations higher than 0.5 mg/dl were excluded from the study. No evidence of bacterial or viral infections was found when blood samples were taken. CD28, CTLA-4 and CD40L expressions were evaluated during stable graft function (with serum creatinine levels ≤1.5 mg/dl) at least 6 months (median: 20 months) after transplantation. The patients were classified into four subgroups based on quartile estimation (25%, 50%, 75%) of the study population's age: (A) 17–35 years, (B) 36–45 years, (C) 46–55 years and (D) 56–68 years. There were no statistically significant differences between the four studied age groups with respect to gender, HLA mismatches, and mean trough levels of cyclosporine A or tacrolimus. An additional analysis comparing clinical and immunological factors in the kidney allograft recipients below 50 and above 50 years of age was performed (Table 1). Both age groups (<50 and >50 years) did not significantly differ in terms of rejection episodes and immunosuppressive regimens (Table 2); however, acute rejections appeared in younger recipients more frequently than in those >50 years (48.2% vs. 27.3%).

Table 1. Clinical and immunological characteristics of the kidney allograft recipients <50 and >50 years of age.

	<50 years mean (±)	>50 years mean (±)	*P*-value
Age (years)	39 (8.4)	55.1 (4.6)	0.0000
Creatinine (mg/dl)	1.58 (0.9)	1.49 (0.7)	0.7990
HLA AB compatible	1.4 (0.7)	1.6 (0.7)	0.2244
HLA DR compatible	1.09 (0.4)	1.04 (0.4)	0.7806
Average PRA (%)	4.7 (10)	5.7 (10)	0.7455
CIT (h)	23.7 (8.0)	25.1 (7.9)	0.5540
CsA dose* (mg)	257 (159)	256 (128)	0.9863
CsA level* (ng/ml)	190 (83)	197 (115)	0.7990
Tacrolimus dose*	8 (4.3)	11 (6.1)	0.2784
Tacrolimus level* (ng/ml)	10.8 (4.2)	15.9 (10)	0.1308
Azathioprine dose (mg)	86 (27)	95 (47)	0.4383
MMF dose (mg)	1620 (545)	1875 (353)	0.2263
Expression of costimulatory molecules			
S-CTLA4	7.8 (10)	3.9 (5)	0.1025
S-CTLA4 st	12.1 (23)	3.8 (4)	0.1261
I-CTLA4	8.2 (19)	4.0 (6.3)	0.3529
I-CTLA4 st	11.8 (20.6)	16.7 (23.7)	0.4022
CD28	80.9 (24)	63.4 (35)	0.0164
CD28 st	65.2 (35)	55.1 (34)	0.2706
CD40	4.4 (4.5)	3.9 (4.4)	0.6684
CD40 st	14.5 (20)	7.7 (10)	0.1529

*Daily total dose/level at the time of measurement of costimulatory molecule expression.

Table 2. Immunosuppressive regimens and rejection episodes in kidney allograft recipients <50 and >50 years of age.

	<50 years *n* = 56	>50 years *n* = 22	*P*-value (Fisher's exact test)
TAC	16	6	0.580
CsA	35	16	0.299
AZA	29	10	0.475
MMF	28	11	0.582
Sir	2	0	–
Rejection episodes (biopsy proven)	27 (48.2%)	6 (27.3%)	0.214

Isolation of cells and conditions

Peripheral blood mononuclear cells (PBMCs) were separated by buoyant density-gradient centrifugation on Lymphoflot (Biotest, Dreieich, Germany) from freshly drawn peripheral venous blood (PB) and washed three times in 0.9% saline. The PBMCs were resuspended at 1×10^6 PBMC/ml in RPMI-1640 medium (Gibco, Paisley, UK) supplemented with 10% fetal calf serum (Flow Laboratories, Irvine, UK), L-glutamine, and 50 mg/ml gentamycin (Gibco) and then cultured with 10 ng/ml anti-CD3 monoclonal antibody (mAb) (Ortho, Neckargemund, Germany) and 500 U/ml rIL-2 (Eurocetus, Amsterdam, The Netherlands). In our experimental model, rIL-2 was used as a second signal to obtain optimal stimulation of T cells [20].

Flow cytometric analysis

All experiments on cells were conducted by triple labelling with PerCP-, FITC-, or RPE-conjugated monoclonal antibodies (MoAbs). The MoAbs used in all experiments were: anti-CD3/PerCP (Becton Dickinson, San Jose, CA, USA), anti-CD4/RPE and anti-CD4/FITC (Becton Dickinson), pure anti-CTLA-4 and anti-CTLA-4/RPE (PharMingen, San Diego, CA, USA), anti-CD40L/RPE (Becton Dickinson) and anti-CD28/FITC (Serotec, Kidlington, UK). As an analysis of the expressions of CD28, CD40L and CTLA-4 molecule within the $CD4^+$ population would be complicated by the fact that the CD4 antigen is also present on the monocyte cell surface, we performed our studies using a triple immunostaining method (a T-cell marker, a T-cell subset (CD4) marker and a CD40L, CD28, sCTLA-4, or iCTLA-4 marker). Briefly, after isolation the cells were washed twice in phosphate-buffered saline (PBS) (without Ca^{2+} and Mg^{2+}) containing 0.5% Tween-20. The cells were then incubated for 30 min with the antibodies described above and excess, unbound antibodies were removed by two washes with PBS. Following these washes, the cells were fixed with 1.5% paraformaldehyde in PBS and analysed by flow cytometry using a FACScalibur flow cytometer (Becton Dickinson). The estimated detection limit of the FACScalibur flow cytometer is 750 molecules of equivalent soluble fluorescein. For intracellular detection of CTLA-4, the cells were first incubated for 30 min at 4 °C in the dark with pure anti-CTLA-4 MoAb to block surface CTLA-4 molecules, then fixed and permeabilized according to a method previously described [21,22], and then stained with CD3/PerCP, CD4/FITC and CTLA-4/RPE antibodies. Negative controls were always performed by omitting the MoAb and by incubating the cells with mouse Ig of the same isotype as the MoAbs conjugated with PerCP, FITC, or RPE. The results were expressed as the proportion of $CD3^+/CD4^+$ cells expressing CTLA-4 (on the cell surface and intracellularly), CD40L, or CD28 molecules. At least 10 000 events per sample were analysed.

Statistical analysis

Analysis was performed using the Kruskal–Wallis test, Mann–Whitney *U*-test, Fisher test and Spearman correlation test in Statistica 6.0 software (StatSoft, Tulsa, OK, USA). The results were expressed as the median and lower and upper quartiles. Differences were considered statistically significant when the *P*-value was <0.05. All the procedures were approved by the Ethics Committee of Wroclaw Medical University.

Results

Expression of costimulatory CD28 molecule on freshly isolated and anti-$CD3^+$rIL-2-stimulated $CD4^+$ T cells

The occurrence of $CD4^+/CD28^+$ cells in freshly drawn PB was significantly higher in younger than in older recipients of a kidney allograft, as presented in Table 3. The

Table 3. The median percentage (and interquartile range) of costimulatory molecules on peripheral $CD4^+$ T cells before and after stimulation (st) with anti-$CD3^+$rIL-2 in the age groups. sCTLA-4 represents surface expression and iCTLA-4 intracellular expression.

Costimulatory molecules	A: 17–35 years (*n* = 16)	B: 36–45 years (*n* = 27)	C: 46–55 years (*n* = 24)	D: 56–68 years (*n* = 11)	*P*-value
S-CTLA4	6 (1.5–12.45)	4.4 (1.8–10.2)	4.2 (1.7–11.9)	2.3 (1.7–3.3)	<0.03 A vs. D
S-CTLA4 st	3.1 (0.65–3.7)	4 (1.5–13.8)	5.4 (1.6–5.5)	4.9 (1–2.4)	0.07 A vs. D
I-CTLA4	3.6 (0.8–5.1)	2 (1.2–7.8)	2 (1–4.1)	1.9 (1.2–3.8)	0.05 A vs. D
I-CTLA4 st	1.4 (2.1–6.6)	4.3 (3–21.1)	2.7 (2.2–12.4)	1.6 (1.3–18.5)	ns
CD28	98 (94–99)	85 (52–97)	69 (42–97)	65 (41–93)	<0.05 A vs. C; < 0.01 A vs. D
CD28 st	88 (40–97)	76 (21–96)	59 (15–94)	48 (21–93)	<0.05 A vs. C; 0.002 A vs. D
CD40	1.85 (1–4.5)	3.2 (0.8–7.1)	2.25 (0.8–3.9)	1.4 (1–3.9)	ns
CD40 st	4.5 (2.1–18.2)	8.7 (5.3–15.8)	4.8 (2.8–9)	4.9 (2.8–12.8)	ns

median percentage of peripheral $CD4^+/CD28^+$ T cells in group A was 98 and in group D was 65 ($P < 0.01$). After stimulation with anti-$CD3^+$rIL-2, the difference in expression of CD28 molecule between groups A and D was even higher (88 vs. 48, $P = 0.002$). Figure 1 shows representative data of the CD28 surface expression on peripheral blood $CD4^+$ T cells in kidney graft recipients.

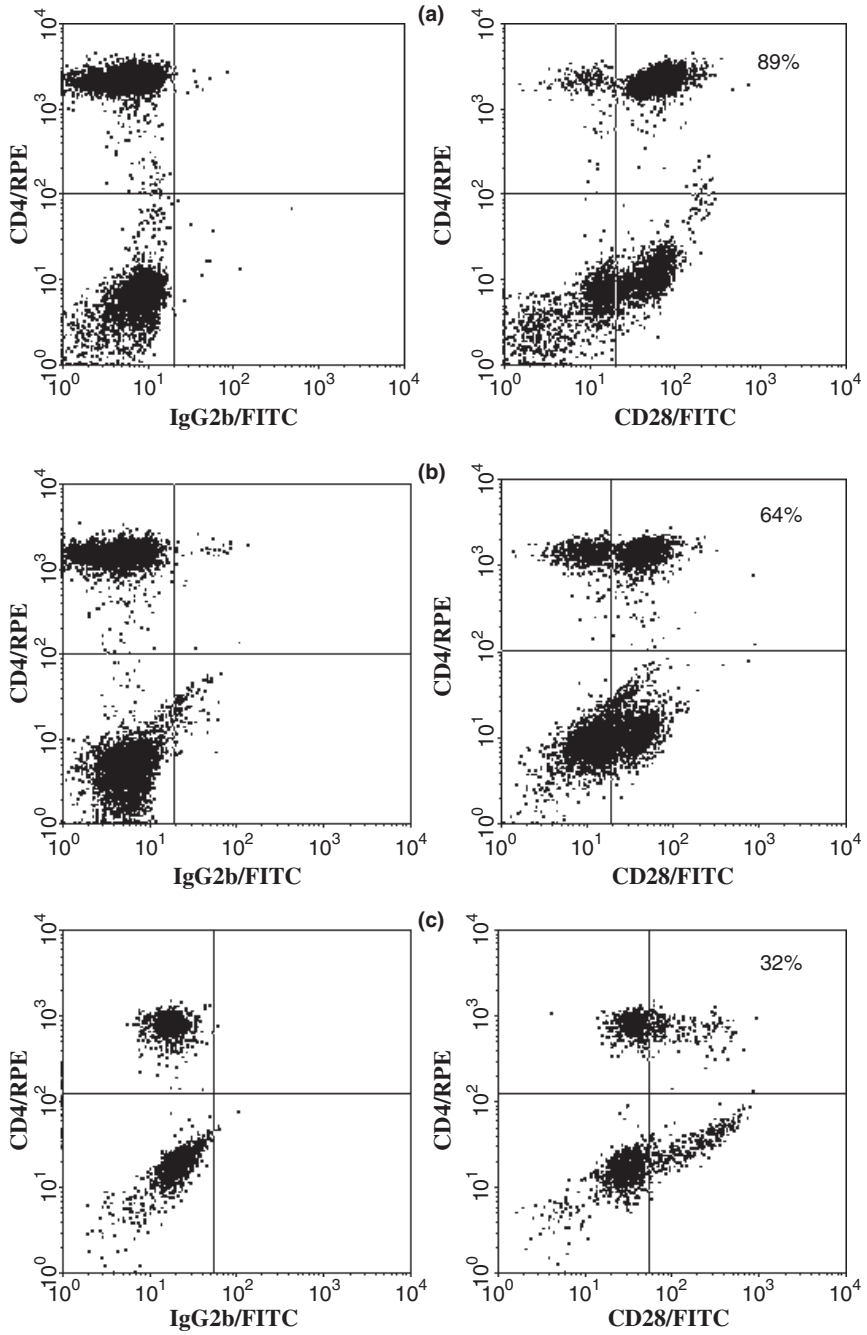

Figure 1 The dot plots show representative data of the CD28 expression on peripheral blood $CD4^+$ T cells from kidney graft recipients with stable graft function. The dot plots show the CD28/FITC vs. CD4/RPE distribution on $CD3^+$ cells. (a) High CD28 expression on freshly isolated $CD4^+$ T cells, (b) Low CD28 expression on freshly isolated $CD4^+$ T cells, (c) Low CD28 expression on $CD4^+$ T after culture with anti-$CD3^+$rIL-2.

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Table 4. Spearman correlation tests between age of kidney transplant recipients and median percentages of $CD4^+$/CTLA-4^+, $CD4^+$/$CD28^+$ and $CD4^+$/$CD40L^+$ T cells before and after stimulation (st) with anti-$CD3^+$rIL-2; sCTLA-4 represents surface expression and iCTLA-4 its intracellular expression.

Correlations (Spearman *R*)	*R*	*P*-value
Age and sCTLA4	−0.094	0.4079
Age and sCTLA4st	0.345	0.0081
Age and iCTLA4	−0.079	0.4911
Age and iCTLA4st	0.110	0.3357
Age and CD28	−0.404	0.0004
Age and CD28 st	−0.146	0.1964
Age and CD40L	0.024	0.8530
Age and CD40L st	−0.015	0.9082

Expression of surface CTLA-4 (sCTLA-4) and intracellular CTLA-4 (iCTLA-4) on freshly isolated and anti-$CD3^+$rIL-2-stimulated $CD4^+$ T cells

A significant difference in the median percentages of $CD4^+$/sCTLA-4^+ T cells in freshly drawn PB was found between groups A and D (6 vs. 2.3, $P < 0.03$) (Table 3). A tendency toward lower expression of $CD4^+$/iCTLA-4^+ T cells in freshly drawn PB was noted in group D compared with group A (1.9 vs. 3.6, $P = 0.05$). In an additional analysis of the two larger groups of recipients (<50 and >50 years), the expressions of both sCTLA-4 and iCTLA-4 on freshly isolated $CD4^+$ T cells did not differ (Table 1). The proportions of $CD4^+$/sCTLA-4^+ and $CD4^+$/iCTLA-4^+ T cells in PB after stimulation with anti-$CD3^+$rIL-2 did not markedly differ between these two age groups.

Expression of the CD40 ligand (CD40L) on freshly isolated and anti-$CD3^+$rIL-2 stimulated $CD4^+$ T cells

Comparing the level of expression of CD40L on freshly isolated cells and in response to anti-$CD3^+$rIL-2 stimulation, no differences were found among the studied groups (Table 3). *Ex vivo* stimulation increased the median percentage of $CD4^+$ T cells expressing CD40L in each age group.

Correlation coefficient

A significant negative correlation between age and the median percentage of peripheral $CD4^+$/$CD28^+$ T cells ($r = -0.4$, $P = 0.0004$) was found in the Spearman correlation test (Table 4). Simultaneously, a significant positive correlation between age and the level of surface expression of CTLA-4 after stimulation with anti-$CD3^+$rIL-2 ($r = 0.34$, $P = 0.008$) was noted. The expression of CD40L on $CD4^+$T was not affected by the age of the recipients.

Table 5. Multivariate regression analysis with the type of immunosuppression and age as dependent variables potentially influencing CD28 expression.

Independent variables influencing CD28 expression	Coefficient	Std. Error	*t*	*P*	correlation coefficients, *r*
Constant	111.565				
Age	−0.934	0.302	−3.091	**0.003**	−0.311
CsA	14.172	8.618	1.645	0.105	0.064
AZA	−10.713	7.557	−1.418	0.161	−0.062
Constant	100.175				
Age	−0.925	0.300	−3.081	**0.003**	−0.311
CsA	14.543	8.495	1.712	0.091	0.064
MMF	11.645	7.456	1.562	0.123	0.086
Constant	125.737				
Age	−0.934	0.302	−3.091	**0.003**	−0.311
TAC	−14.172	8.618	−1.645	0.105	−0.064
AZA	−10.713	7.557	−1.418	0.161	−0.062
Constant	114.717				
Age	−0.925	0.300	−3.081	**0.003**	−0.311
TAC	−14.543	8.495	−1.712	0.091	−0.064
MMF	11.645	7.456	1.562	0.123	0.086

Influence of immunosuppression on costimulatory molecules expression

No expression differences in any of the measured costimulatory molecules were noted when comparing stable transplant recipients who had received different immunosuppressive regimens (CsA + AZA versus CsA + MMF versus TAC + Aza versus TAC + MMF, data not shown). A multivariate regression analysis with the type of immunosuppression and age as dependent variables was performed to determine the impact on CD28 expression. Age remained an independent factor influencing CD28 molecule expression in all the immunosuppressive regimens used (Table 5).

Discussion

Recent reports have shown no differences in graft or death-censored graft survival between kidney allograft recipients over 60 and those below 60 years of age [23,24]. Available databases clearly show that the major causes of graft loss in elderly allograft recipients are chronic complications (cardiovascular, malignancies, infections) and not rejection [1,4,7].

Clinical studies suggest that older transplant recipients have reduced acute allograft rejection rates and that in the older population it appears that the intensity of the rejection process is weaker than in younger recipients. This may result from the fact that an immunocompetent,

aging individual becomes more tolerogenic to alloantigen. There is, however, a growing body of evidence showing that an allograft from an older donor transplanted to a younger recipient results in a more intensive immune response [25]. This was observed in the early post-transplant period and may influence long-term outcome. Orsenido *et al.* analysed both recipient and donor ages as factors influencing patient and graft survival after kidney transplantation [26]. The overall rates of patient and kidney graft survival were comparable to those of younger patients. Only donor age ≥55 years had a negative effect on patient and kidney graft survival.

More recently we have shown that kidney allograft recipients with uneventful post-transplant course, lasting at least 18 months, with stable graft function (serum creatinine ≤1.5 mg/dl) have enhanced surface CTLA-4 and intracellular CTLA-4 expression on freshly drawn $CD4^+$ T cells in comparison with recipients with chronic allograft nephropathy [27]. Additionally, these patients with stable graft function exhibited a higher potential to express surface CTLA-4 in response to *ex vivo* stimulation.

In this study, we demonstrated that the expression of costimulatory and inhibitory molecules on T cells changes with age in kidney allograft recipients. *Ex vivo* stimulation resulted in a significant decrease in the frequency of $CD28^+/CD4^+$ T cells in all groups (A–D). It was previously established that CD28 expression is physiologically down-regulated after 24 h at both the mRNA and protein levels and returns to prestimulation levels thereafter. Such was already observed in recipients with stable graft function [27].

We found a lower frequency of $CD4^+/CD28^+$ cells in freshly drawn PB from older kidney allograft recipients. Moreover, after stimulation with anti-$CD3^+$rIL-2 the differences in the median percentages of $CD28^+$ T cells among the age groups were higher than without stimulation. This may account significantly for the age-related diminution of T-cell response to mitogenic signals.

Many studies on the mechanism of aging have shown that the number of $CD28^+$ T cells decreases with age. CD28, the major T-cell-specific costimulatory molecule, is expressed on nearly 100% of human T cells at birth [28]. During life the human body accumulates memory $CD8^+$ T cells that are CD28-negative, with some elderly persons having more than 50% of their total $CD8^+$ T-cell pool being CD28-negative [29,30]. This change in the composition of the memory T-cell pool is of fundamental importance, as CD28 has been implicated in a multitude of critical T-cell functions, including IL-2 gene transcription, apoptosis, stabilization of cytokine mRNA and cell adhesion [31–33].

The molecular basis for the loss of CD28 expression on $CD4^+$ T cells has been proposed to be associated with silencing of the CD28 gene transcriptional initiator (INR) [34]. These T cells may exhibit reduced vaccine responsiveness and may exhibit suppressor function [35]. Efforts to understand the mechanism responsible for this specific gene repression event will provide insights into both T-cell biology as well as possible immunotherapeutic approaches.

A slight decrease in the median percentages of $CD4^+/sCTLA\text{-}4^+$ T cells in freshly drawn PB was observed in older recipients. Although stimulation with anti-$CD3^+$rIL-2 showed different expressions of sCTLA-4 and iCTLA-4 molecule among the studied age groups, a tendency to higher expression was observed in group D (56–68 years). The latter observation may result from the hyporesponsiveness of T cells in older patients. Its contribution to diminished responsiveness of T cells is based on observations that CTLA-4 downregulates T-cell responses by raising the threshold for effective T-cell activation and that very high CTLA-4 levels result in anergy [13,36]. As CTLA-4 expression is dependent on CD28 engagement, the question arose whether the deficiency in CD28 influences the ability to upregulate CTLA-4. In our study the significant negative correlation between age and the percentage of $CD28^+$ T cells corresponded with a positive correlation between age and the expression of CTLA-4 after stimulation with anti-$CD3^+$rIL-2. This finding supports the observation that chronic T-cell stimulation is less CD28 dependent. Leng *et al.* reported a decrease in $CD8^+/CD28^+$ T cells counts ($R = -0.67$, $P < 0.001$) with age and a simultaneous positive correlation between age and the percentage of $CD4^+/CTLA\text{-}4^+$ T cells ($R = -0.6$, $P < 0.001$) [19]. In contrast to our results, they received stronger coefficients, which may result from the broader age range of the investigated healthy individuals (18–94 years). Moreover, the weaker coefficients in our analysis could be a consequence of immunosuppressive treatment.

We believe that diminished expression of CD28 accompanied by increased expression of sCTLA-4 after stimulation may play a role in the immunoregulatory activity mediated by some $CD4^+$ T cells during immune insult. CD28 functions as a regulator of T-cell responses and induces a balance of signals enhancing and regulating T-cell responses. As CTLA-4 is not detectable on naive T cells, the expression of both molecules, surface and intracellular CTLA-4, on peripheral blood $CD4^+$ T cells indicates the involvement of $CD4^+$ T cells in an ongoing immune response. CTLA-4 functions by out-competing CD28 for their common ligand due to its higher affinity. As a result, one of the mechanisms of the alloimmune insult can be stopped and a favourable post-transplant course achieved.

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The expression of CD40L on freshly isolated cells and in response to anti-CD3$^+$rIL-2 stimulation did not differ among the studied groups. As *ex vivo* stimulation did increase the median level of CD40L expression in all age groups, we assume that these cells were not stimulated *in vivo* and do respond to re-stimulation with rIL-2. Fernandez-Gutierrez *et al.* reported impaired CD40L expression in elderly human T cells following anti-CD3 challenge. However, activation by PMA and/or ionomycin was preserved both in T cells (CD40L expression) and in B cells (CD23 expression) [37].

The lack of characteristics of the T cells infiltrating the graft is a limitation of this study. However, we believe that the significantly diminished frequency of CD28-positive cells is of note and of potential clinical significance. The CD28 molecule is constitutively expressed in CD4 and CD8 positive lymphocytes and its stimulation is required for effective T-cell response initiation. Therefore our observation adheres to the well-proven clinical data showing that the main cause of graft lost in the >55-year-old recipient is, besides cardiovascular diseases, infection, but not rejection. It seems reasonable to assume that the decrease in CD28-positive lymphocyte counts is a hindrance to the mounting of an effective response against donor antigens (a beneficial aspect, but in the presence of an infectious agent a drawback).

We conclude that the distinct pattern of costimulatory molecule expression, i.e. diminished expression of CD28 molecules and an increased ability for poststimulation CTLA-4 expression, are associated with age (immune senescence) and may be helpful in transplant acceptance. Our data seem to support the notion that the long-term intensity of an immunosuppressive regimen can be safely diminished in older kidney allograft recipients with uneventful course.

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Authorship

MK: designed study, collected data, analyzed data, wrote the paper. AK: performed study, analyzed data. MM-P: designed study, collected data. IF: performed study, contributed important reagents. LC: performed study. DB: performed study. DP: contributed important reagents. MK: contributed important reagents, critical revision of the manuscript for important intellectual content.

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