Peripheral natural killer cell and allo-stimulated T-cell function in kidney transplant recipients associate with cancer risk and immunosuppression-related complications

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Reducing immunosuppression has been proposed as a means of preventing cancer in kidney transplant recipients but this can precipitate graft rejection. Here we tested whether anti-tumor natural killer (NK) cell and allo-responsive T-cell function in kidney transplant recipients may predict cancer risk and define risk of rejection. NK cell function was measured by the release of lactate dehydrogenase and T-cell allo-response by interferon-γ quantification using a panel of reactive T-cell enzyme-linked immunospot (ELISPOT) in 56 kidney transplant recipients with current or past cancer and 26 kidney transplant recipients without cancer. NK function was significantly impaired and the allo-response was significantly lower in kidney transplant recipients with cancer. With prospective follow-up, kidney transplant recipients with poor NK cell function had a hazard ratio of 2.1 (95% confidence interval 0.97–5.00) for the combined end point of metastatic cancer, cancer-related death, or septic death. Kidney transplant recipients with low interferon-γ release were also more likely to reach this combined end point. Thus, posttransplant monitoring of allo-immunity and NK cell function is useful for assessing the risk of over immunosuppression for the development of malignancy and/or death from cancer or sepsis.

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Kidney transplant recipients (KTR) treated with immune suppressants have a 3- to 5 -fold increased risk of developing solid organ cancers and a 60- to 200-fold increased risk of developing squamous cell carcinoma (SCC) over the general population.[1–6] Malignancies that develop in KTR taking immune suppressants are more aggressive, more likely to recur, and have poorer prognoses compared with cancers that occur in immune-competent subjects.[2,3,7] In addition, KTRs who develop multiple SCCs are at increased risk of developing subsequent *de novo* solid organ cancer compared with KTRs who never develop SCCs.[8]

The known association between the risk of malignancy and chronic immunosuppression in KTR have resulted in attempts to reduce immunosuppressive drugs, as guidelines suggest.[9] This includes reduction in calcineurin inhibitor (CNI) levels and/or conversion to drug regimens containing mammalian target of rapamycin inhibitors, the latter having direct anti-tumor effects.[10–12] Although some studies have indicated that immunosuppression reduction is associated with a reduced incidence of cancer in KTRs,[10–12] findings do not uniformly support a protective effect[12–14] and immunosuppression withdrawal can increase the risk of developing acute cellular and/or antibody-mediated rejection.[11,12] As a consequence, identifying and validating biomarkers capable of accurately delineating risk of rejection and malignancy in KTRs has become a priority. With such biomarkers in hand, it would be possible to individualize immunosuppression so as to limit anti-donor allo-immunity sufficiently to prevent rejection, while simultaneously leaving sufficient anti-tumor immunity intact so as to prevent development of posttransplant malignancy.

Measurement of cellular allo-immunity in KTRs has the potential to inform clinicians sabout risks of rejection and malignancy. Previous studies have shown the development of a panel of reactive T cell (PRT) interferon-γ (IFN-γ) enzyme-linked immunospot (ELISPOT) assay, which quantifies the frequency of primed/memory T cells in the peripheral blood

reactive to a panel of allogeneic stimulator cells expressing multiple human leukocyte antigen (HLA) molecules. Evidence indicates that positive PRT ELISPOT results identify pre-transplanted KTR at risk for developing acute cellular rejection.[15,16]

Humoral-mediated rejection risk can be determined by measuring levels of *de novo* donor-specific antibodies (DSAs),[17] which target donor HLA, and together these assays may provide enough evidence to determine how strongly primed the immune system is and how high rejection risk is, before drug manipulations in KTRs with cancer.

As accumulating evidence suggests that natural killer (NK) cells confer anti-tumor immunity, we postulated that one potentially informative biomarker for assessing risk of developing posttransplant malignancy is measurement of the immune function of NK cells. Although NK cell numbers in KTRs with malignancy have been investigated,[18,19] NK cell function has not been extensively studied in KTRs.[20] The measurement of lactate dehydrogenase released from lysed NK-sensitive myelogenous leukemia cell line K562 can be used to determine NK function[21] and may provide evidence of a KTR's ability to clear malignancy.

As a first step toward developing a multifaceted monitoring approach for balancing risks of rejection and malignancy, we performed a cross-sectional observational study using a cohort of KTR with and without malignancies using PRT ELISPOT, DSA, and functional NK cell assays. Our results support the hypothesis that these assays have the potential to guide drug manipulation tailored to the transplant recipient's immune biological function rather than based solely on drug levels.

RESULTS

In an effort to identify biomarkers that differentiate KTRs with and without malignancy, we compared immune parameters including DSA, NK cell function, and primed T-cell allo-immunity in 82 KTRs: 31 KTRs with current cancer, 25 KTRs with past cancer, and 26 control KTRs (without current or past cancers). The age and duration of immunosuppression were greater in KTRs with past cancer than other KTRs (Table 1). However, immunosuppressive drug doses and serum levels did not differ among the groups nor did gender, serum creatinine, rejection episodes, multiple grafts, or NK cell number (Table 1). Fewer KTRs with cancer were administered mycophenolate- or

Table 1 | Cohort demographics and clinical parameters

	Current cancer	Past cancer	No cancer	*P*-value
Numbers (*N*)	31	25	26	
Age, median (range)	63 (43–75)	60 (44–73)	58 (39–76)	0.044
Male gender, *N* (%)	24 (77)	19 (76)	16 (62)	0.357
Years immunosuppressed, median (range)	9 (4–35)	14 (4–35)	7 (2–31)	0.006
Serum creatinine, median (range)	117 (66–330)	123 (65–202)	108 (51–324)	0.983
eGFR, median (range) ml/min/1.73 m^2	60 (20–60)	44 (15–60)	50 (15–60)	0.063
Presence of proteinuria	2 (7)	8 (32)	8 (31)	0.020
Rejection episode(s), *N* (%)	5 (16)	3 (12)	9 (35)	0.100
Multiple grafts, *N* (%)	5 (16)	2 (8)	5 (19)	0.503
DSA >1500 MFI, *N* (%)	5 (16)	3 (12)	7 (27)	0.358
Biopsy-proven transplant glomerulopathy	3 (10)	5 (20)	4 (15)	0.546
NK cells/μl of blood, median (range)	97 (1–1009)	153 (23–377)	86 (4–242)	0.534
Immunosuppressive drug regimen				
Azathioprine, *N* (%)	7 (23)	5 (20)	3 (12)	0.542
Mycophenolate, N (%)	13 (42)	16 (64)	22 (85)	0.004
Calcineurin inhibitors, *N* (%)	15 (48)	13 (52)	20 (77)	0.068
mTORi, *N* (%)	8 (26)	4 (16)	3 (12)	0.358
Prednisolone, *N* (%)	27 (87)	20 (80)	22 (85)	0.700
Triple therapy, *N* (%)	12 (39)	11 (44)	18 (69)	0.056
Immunosuppressive drug dose				
Azathioprine, median (range), mg	100 (12.5–100)	25 (25–100)	100 (50–100)	0.516
Mycophenolate, median (range), g	1 (0.5–1.5)	1 (1–2)	1.25 (0.5–2)	0.162
Cyclosporine, median (range), mg	150 (150, 150)	100 (50–250)	125 (80–200)	0.855
Tacrolimus, median (range), mg	4 (1–14)	4 (3–8)	4 (1.5–8)	0.925
mTORi, median (range), mg	2 (1.5–3)	3 (1.5–3)	2 (1.5–4)	0.670
Prednisolone, median (range), mg	5 (2.5–10)	5 (4–10)	5 (5–10)	0.241
Immunosuppressive drug levels				
Tacrolimus, median (range), μg/l	5.5 (2–11)	4 (3–13)	7 (3–13)	0.813
mTORi, median (range), μg/l	7.5 (3–15)	6 (5–13)	2 (1.5–4)	0.069

Abbreviations: DSA, donor-specific antibody; eGFR, estimated glomerular filtration rate; MFI, mean fluorescence intensity; mTORi, mammalian target of rapamycin inhibitor; NK, natural killer.
Underlined values indicate $P<0.05$.