Donor-specific hypo-responsiveness occurs in simultaneous liver-kidney transplant recipients after the first year

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Kidney allografts of patients who undergo simultaneous liver-kidney transplantation incur less immune-mediated injury, and retain better function compared to other kidney allografts. To characterize the host alloimmune responses in 28 of these patients, we measured the donor-specific alloresponsiveness and phenotypes of peripheral blood cells after the first year. These values were then compared to those of 61 similarly immunosuppressed recipients of a solitary kidney or 31 recipients of liver allografts. Four multicolor, non-overlapping flow cytometry protocols were used to assess the immunophenotypes. Mixed cell cultures with donor or third party cells were used to measure cell proliferation and interferon gamma production. Despite a significant overlap, simultaneous liver-kidney transplant recipients had a lower overall frequency of circulating $CD8^+$, activated $CD4^+$ and effector memory T cells, compared to solitary kidney transplant recipients. Simultaneous liver-kidney transplant recipient T cells had a significantly lower proliferative response to the donor cells compared to solitary kidney recipients (11.9 vs. 42.9%), although their response to third party cells was unaltered. The frequency of interferon gamma producing alloreactive T cells in simultaneous liver-kidney transplant recipients was significantly lower than that of solitary kidney transplant recipients. Flow cytometric analysis of the mixed cultures demonstrated that both alloreactive $CD4^+$ and $CD8^+$ compartments of the simultaneous liver-kidney transplant recipient circulating blood cells were smaller. Thus, the phenotypic and functional characteristics of the circulating blood cells of the simultaneous liver-kidney transplant recipients resembled those of solitary liver transplant recipients, and appear to be associated with donor-specific hypo-alloresponsiveness.

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Compared with solitary kidney transplantation (KTA), the incidences of both T-cell–mediated and antibody-mediated rejection of the renal allograft are lower after simultaneous liver-kidney transplantation (SLK).[1] The protective effect of the liver allograft appears to be active in the long term and depends on the quality and function of the liver. Furthermore, the incidence of chronic subclinical inflammation in the kidney allograft of SLK recipients is remarkably lower and is associated with preservation of kidney function.[1]

Recently, we demonstrated that the molecular markers of inflammation and T-cell activation are significantly less common in kidney biopsy specimens of SLK recipients compared with KTA recipients.[2] In addition, expression of the genes associated with tissue integrity and metabolism are upregulated in the renal allografts of SLK recipients. Whether these molecular changes are associated with overall differences in the alloimmune responses in the 2 cohorts or related to improved tissue repair mechanisms in the SLK recipients remains unknown. Thus, the goal of the current study was to investigate the donor-specific alloresponse in SLK recipients after the first year and to examine the immunophenotypes of peripheral blood cells compared with those of similarly immunosuppressed KTA recipients. Results were also compared with solitary liver transplant (LTA) recipients.

RESULTS

Recipient and donor characteristics

A total of 63 KTA, 28 SLK, and 34 LTA recipients with available cryopreserved peripheral blood mononuclear cells (PBMCs) were identified. As the goal of the study was to compare the systemic alloresponses at steady state, 2 KTA and 3 LTA recipients were excluded because of either a histologically documented rejection episode within 1 year of the sample collection or severe bacterial, viral, or fungal infection within 6 months of the sample collection. The resulting 61 KTA, 28 SLK, and 31 LTA recipient samples were included in the analyses (Figure 1). The donor and recipient characteristics of the groups are summarized in Table 1. Briefly, there were no differences in either the recipient or donor sex and race between the groups. Overall, the SLK recipients were younger ($P = 0.007$ vs. KTA and $P = 0.02$ vs. LTA). The average human leukocyte antigen (HLA) mismatch was higher among the SLK and LTA recipients (4.7 ± 1.0 and

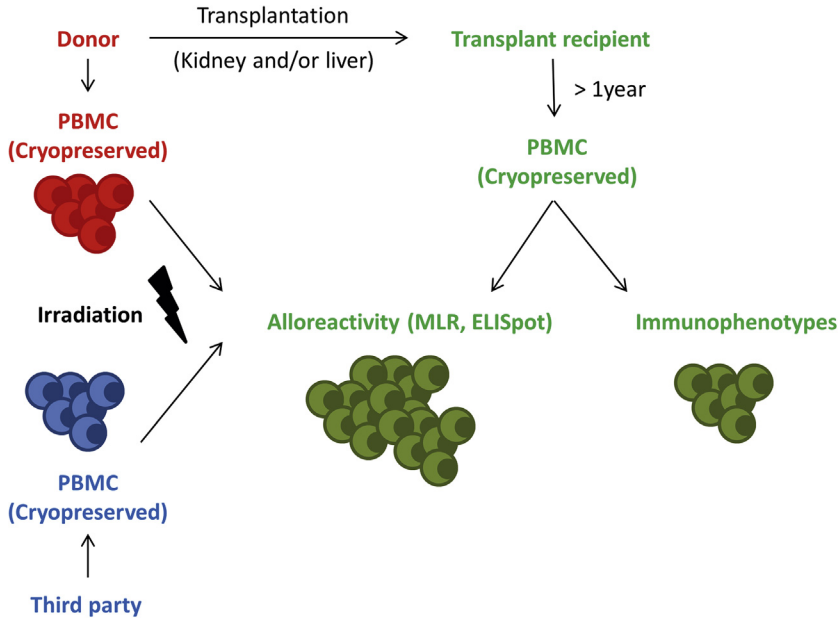

Figure 1 | Study design and experimental setups. Cryopreserved peripheral blood mononuclear cells (PBMC) from transplant recipients (green) were assessed for immunophenotypes and tested for alloreactivity. For the latter, donor (red) or third-party (blue) PBMCs were used as stimulators. MLR, mixed leukocyte reaction.

4.8 ± 0.9, vs. 3.4 ± 1.6; $P = 0.0003$ and $P = 0.0001$, respectively). Per our center's immunosuppression protocol, the majority of the KTA recipients had received thymoglobulin induction, whereas neither SLK nor LTA recipients had T-cell depletion. The PBMC samples were collected at least 1 year after the transplantation (range, 1–4 years, similar in all groups). At the time of the sample collection, the mean trough tacrolimus level was higher among the KTA recipients (7.6 ± 1.3 vs. 6.4 ± 1.6 in SLK and 5.6 ± 1.6 in LTA; $P = 0.0006$ and $P = 0.0001$, respectively). All transplants, with the exception of 1 LTA, were done with a negative flow cytometric cross-match. Details of the donor-specific HLA antibodies (pretransplantation and at the time of the collection of PBMCs) are presented in Supplementary Table S1.

Immunophenotype of PBMCs

The viability of cryopreserved PBMC samples after thawing was consistently >80% and was not different between the 2 groups. The immunophenotypes were analyzed by 10-color flow cytometric protocols that were developed by our group and verified in healthy controls and cancer patients.[3] The protocol for the nonoverlapping leukocyte populations is shown in Supplementary Table S2. The total number of cells (per microliter) was similar between the KTA, SLK, and LTA recipients (Table 2). Similarly, the total number of monocytes and lymphocytes was comparable among the 3 groups. In the lymphocyte compartment, the percentage of B cells (17.7 ± 12.9 vs. 14.2 ± 7.6; $P = 0.34$), T cells (55.5 ± 21.7 vs. 62.1 ± 12.3; $P = 0.28$), and NK cells (18.9 ± 14.5 vs.

Table 1 | Demographic and basic immunologic parameters of the solitary kidney (KTA), liver (LTA), and simultaneous liver-kidney (SLK) transplant recipients

				P		
	KTA ($N = 61$)	SLK ($N = 28$)	LTA ($N = 31$)	KTA versus SLK	SLK versus LTA	KTA versus LTA
Recipient						
Age, yr, ±SD	53.5 ± 14.0	43.6 ± 17.9	53.6 ± 10.6	*0.007*	*0.02*	0.97
Sex (male:female)	36:19	17:11	14:11	0.81	0.78	0.46
Race (Caucasian:non-Caucasian)	53:2	26:2	22:3	0.60	0.66	0.17
Donor						
Age, yr, ± SD	45.1 ± 14.1	40.6 ± 13.5	51.3 ± 15.4	0.17	*0.01*	0.08
Sex (male:female)	28:33	14:14	16:15	0.82	1.00	0.66
Race (Caucasian:non-Caucasian)	55:6	27:1	29:2	0.42	1.00	0.71
HLA mismatch, mean ± SD	3.4 ± 1.6	4.7 ± 1.0	4.8 ± 0.9	*0.0003*	*0.72*	*0.0001*
Induction with thymoglobulin, *N*	43	0	0	*0.0001*	*1.00*	*0.0009*
Trough tacrolimus level, mean ± SD	7.6 ± 1.3	6.4 ± 1.6	5.6 ± 1.6	*0.0006*	*0.08*	*0.0001*

HLA, human leukocyte antigen.
The italics designate significant *P* values (i.e., $P < 0.05$).