Evidence of Immunologic Dysfunction in Older Solid Organ Transplant Recipients

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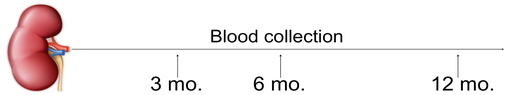
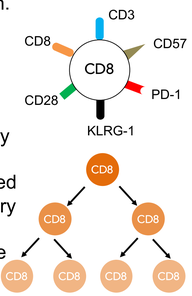
BACKGROUND

- The numbers of older patients with end-stage organ disease requiring organ transplantation continues to grow as the population ages. Compared to younger patients, older transplant recipients experience increased rates of infection and death, but decreased rates of rejection. This observation suggests that immune dysfunction in older transplant recipients leads to vulnerability to infection.
- Age-related immune changes in the healthy older population include immunosenescence (CD28-, KLRG-1+), increased maturation phenotype, and increased frequency of activated (CD57+) and exhausted (KLRG-1+, PD-1+) T cells. This T cell dysfunction contributes to "inflammaging," or the chronic inflammation associated with age-related disease, and is possibly driven by chronic cytomegalovirus (CMV) infection. Changes in innate cell function have also been observed.
- **Hypothesis**: *Compared to younger transplant recipients, older kidney transplant recipients will demonstrate increased immunosenescence, exhaustion, and terminal differentiation; decreased T cell lymphoproliferative ability; and increased pro-inflammatory cytokine and chemokine secretion.*

METHODS



- **Patient characteristics**: Peripheral blood mononuclear cells were isolated from 23 older (≥ age 60) and 35 matched younger (ages 30-59) kidney transplant recipients at 3 months after transplantation. Older versus younger patients were matched on induction type (basiliximab versus lymphocyte depletion with antithymocyte globulin (ATG)) and living versus deceased donor type.
- **Aim 1:** *Evaluation of immune phenotype and function by flow cytometry.* Immunophenotyping was performed by multiparameter flow cytometry using a cocktail of 12 fluorochrome-conjugated antibodies, and proliferation assays were performed. Fluorescence was measured by flow cytometry using the LSRFortessa (BD Bioscience) and analyzed using FlowJo software.
- **Aim 2:** *Evaluation of T cell lymphoproliferative ability.* PBMCs were labeled with CFSE and incubated at 37C for 4 days with PHA (5 ug/mL). Proliferation was measured as loss of CFSE by flow cytometry using the LSRFortessa (BD Bioscience) and analyzed using FlowJo software.
- **Aim 3:** *Quantification of cytokine and chemokine release*. Multiplex cytokine assays (Luminex) were performed on patient serum.



- **Statistical analysis**: JMP Pro 11 was utilized to calculate Wilcoxon test for numerical data and 2-tailed Fisher's exact test chi-square analysis for categorical data. GraphPad Prism was utilized to calculate unpaired t-test for the proliferation data. R was utilized to generate the heat map for the cytokine and chemokine data.

	Older (n = 23)	Younger (n = 35)	p-value
Acute cellular rejection (%)	8.7	17.1	0.458
Antibody mediated rejection (%)	0.0	5.7	0.513
CMV antibody positive (%)	78.3	70.6	0.558
CMV viremia in 1st year (%)	47.8	26.5	0.157
BK viremia in 1st year (%)	30.4	18.2	0.344
Invasive infection in 1st year (%)	17.4	14.3	1.000
Severe infection in 1st year (%)	13.0	14.3	1.000
Death in 1st year (%)	4.3	0.0	0.397

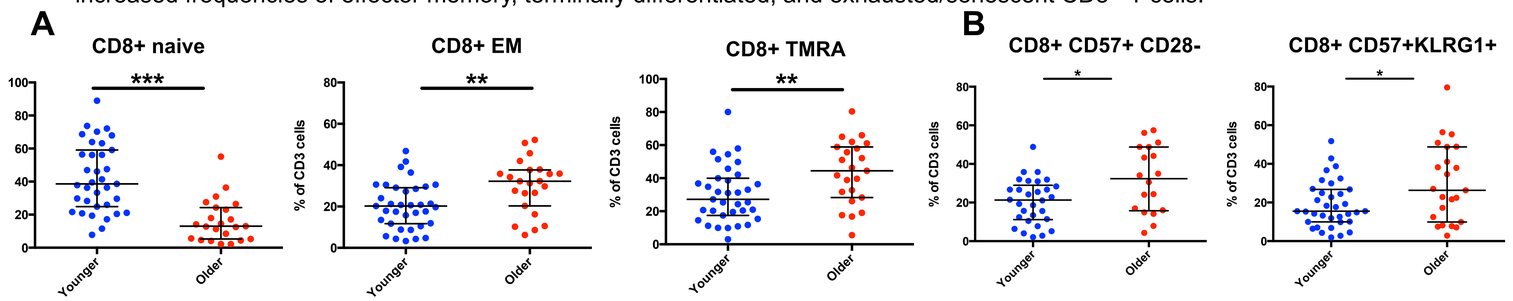
Table 1: Characteristics of older versus younger kidney transplant recipients. Patients were matched by induction and donor type.

	Older (n = 23)	Younger (n = 35)	p-value
Age (median, years)	67	42	N/A
Sex (% female)	26.1	42.9	0.267
Non-white (%)	34.8	34.3	1.000
Hispanic (%)	34.8	37.1	1.000
Diabetes (%)	56.5	29.4	0.056
Induction by lymphocyte depletion (%)	30.4	25.7	0.768
Deceased donor (%)	43.5	42.9	1.000
Time post-transplant (median, days)	89	87	0.084

Table 2: Clinical outcomes of older versus younger kidney transplant recipients.
N/A: Not analyzed as groups are defined by age.

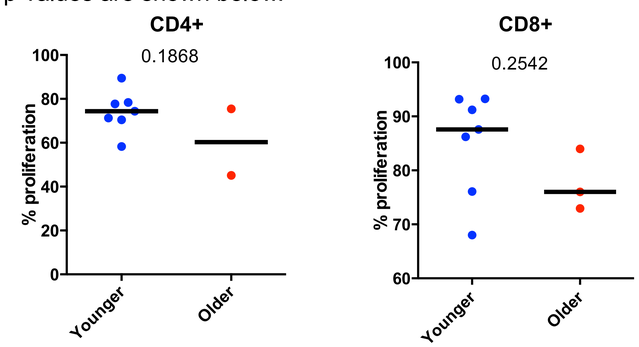
RESULTS

- **Evaluation of immune phenotype by flow cytometry:** Older patients displayed decreased frequencies of naïve CD8+ T cells, and increased frequencies of effector memory, terminally differentiated, and exhausted/senescent CD8+ T cells.

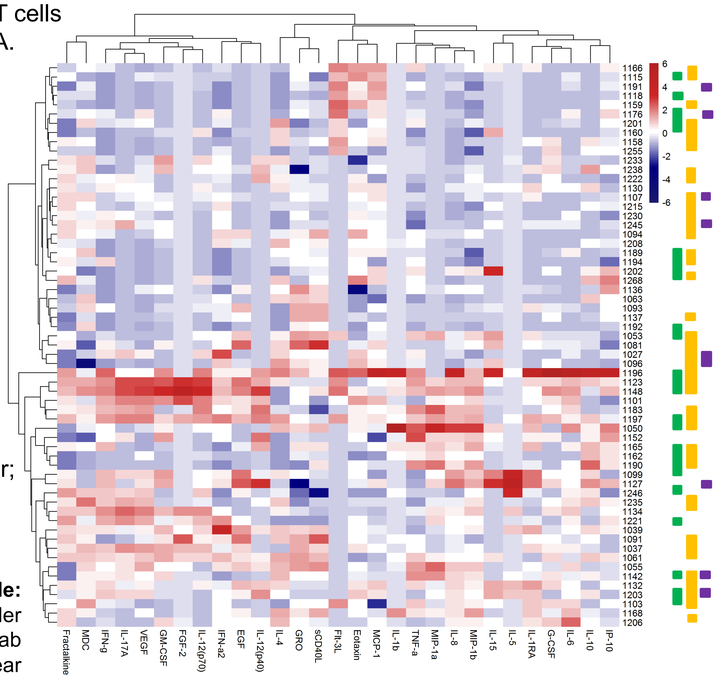


A) Maturation phenotype of older and younger transplant recipients for CD8+ T cells demonstrating frequencies of naïve, effector memory, and terminally differentiated effector cells (TMRA). *** indicates p<0.001, ** p<0.01, * p<0.05. **B)** Immunophenotype of older and younger transplant recipients demonstrating frequencies of senescent (CD57+/CD28-) and exhausted (CD57+/KLRG1+) CD8+ T cells. Total CD8+ CD57+ and KLRG1+ T cells were also more frequent in older patients (data not shown).

- **Evaluation of T cell lymphoproliferative ability:** CD4+ and CD8+ T cells of older patients exhibited decreased proliferation in response to PHA. p-values are shown below.



- **Quantification of cytokine and chemokine release:** Patients who secreted higher levels of cytokines and chemokines clustered together; this zone of the heat map tended to be enriched with older patients. Older patients secreted higher levels of TNFa and FLT-3L, while younger patients secreted higher levels of fractalkine, MDC, and EGF (data not shown).



Heat map color code:
Green: older
Gold: induction with basiliximab
Purple: presence of invasive infection in 1st year

CONCLUSIONS

- A decreased frequency of CD8+ naïve T cells and increased frequencies of effector memory, terminally differentiated, and exhausted/senescent CD8+ T cells were observed in older patients.
- CD4+ and CD8+ T cells of older patients tended to proliferate less than those of younger patients.
- Older patients secreted higher levels of pro-inflammatory cytokines and chemokines.
- These findings suggest a possible mechanism for increased vulnerability to infection and death in the older transplant recipient.
- Further studies, including analysis of changes in gene expression and DNA methylation, will be used to develop a model of immune dysfunction and may lead to noninvasive techniques for monitoring, customization of immune suppression, and candidate selection based on biologic, rather than chronologic, age.

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