

Potential Role for T-Cell Intrinsic DNA Repair in Human Kidney Transplant Rejection

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Disclosures: None



PURPOSE

The spatial location of infiltrating immune cells in kidney allografts, predominately T cells, is diagnostic for rejection and prognostic for graft longevity, though little is known about the functional characteristics of these infiltrating T cells. DNA repair plays a crucial role in maintaining chromatin integrity as T cells migrate through parenchymal tissues. Recent work in small animal models points to a DNA repair enzyme, DNA-dependent protein kinase catalytic subunit (DNA-PKcs), as playing a key role in T cell mediated graft rejection, as treatment with an FDA-approved inhibitor of DNA-PKcs prevented recipient T cell accumulation within allografts though showed no significant impact on the numbers of circulating T cells ¹. Therefore, we sought to characterize the expression of DNA-PKcs and other functional phenotypic markers in graft-infiltrating CD8 T cells in human kidney allografts.

METHODS

Quantitative multiplex immunofluorescence microscopy (mIF) with cell-based segmentation and spatial analysis was performed on multispectral whole slide scans of single tissue sections of archived diagnostic FFPE kidney allograft needle core biopsies. The biopsy cohort was selected on the basis of varying degrees of tubulointerstitial inflammation: acute tubular injury (ATI, n=9); Borderline (Suspicious) for Acute T-cell Mediated Rejection (BL, n=10); and tubulointerstitial-type T-cell Mediated Rejection, Banff grade 1A/1B (TCMR, n=10). Immunolabeling of T cell phenotypic markers including DNA-PKcs, CD3, CD8, TCF1, Ki67, and cleaved caspase 3 as well as a DAPI nuclear stain was performed on a Leica BondRx autostainer with Opal TSA-based detection of the 6-target, 7-color mIF panel (**Table 1**). Following multispectral image acquisition (Phenolmager HT) and spectral unmixing (InForm), cortical regions were manually annotated, and cell-based image analysis was performed using Halo AI (Indica Labs) and the HighPlexFL module (**Figure 1**). Resulting single cell data were exported as a csv file and analyzed in R for intragraft T cell population characteristics across histologic groups using multiple spatial statistical approaches (**Table 2 & Figure 2**).

Table 1. Multiplex Immunofluorescence Staining Protocol

Antigen	1° Antibody	Opal	Pseudocolor
Nuclei	n/a	DAPI	Blue
Cleaved Caspase-3	Cell Signaling (9579)	480	Cyan
CD8	Sigma (SP16)	520	Green
DNA-PKcs	Sigma (HPA035174)	570	Yellow
CD3	Leica (LN10)	620	Orange
TCF1	Cell Signaling (2203)	690	Magenta
Ki67	AbCam (B56)	780	

RESULTS

The density of CD8+ T cells in the renal cortex increased across histologic groups, from ATI to BL to TCMR (**Figure 2**), not unexpected due to Banff diagnostic criteria². With respect to the total cell population in the renal allograft cortex, the proportional positivity of Ki67, a marker of recent cell division, and that of TCF1, a stem-like memory marker in CD8 T cells, were significantly higher in the TCMR group compared to ATI or BL, whereas DNA-PKcs proportional positivity was significantly higher in ATI as compared to BL or TCMR (**Table 2**). DNA-PKcs staining was noted to be bright and diffuse in renal tubular epithelial nuclei in ATI, similar to that seen in other models of acute kidney injury³. Interstitial CD8+ T cells positive for DNA-PKcs were noted across all histologic groups (**Figure 2**), supporting earlier findings of the role of DNA-PKcs in activated T cells⁴. Furthermore, the proportion of CD8 T cells positive for DNA-PKcs was significantly higher in ATI and showed a dynamic and inverse relationship with respect to increasing T cell density in the allograft cortex (**Figure 2**).

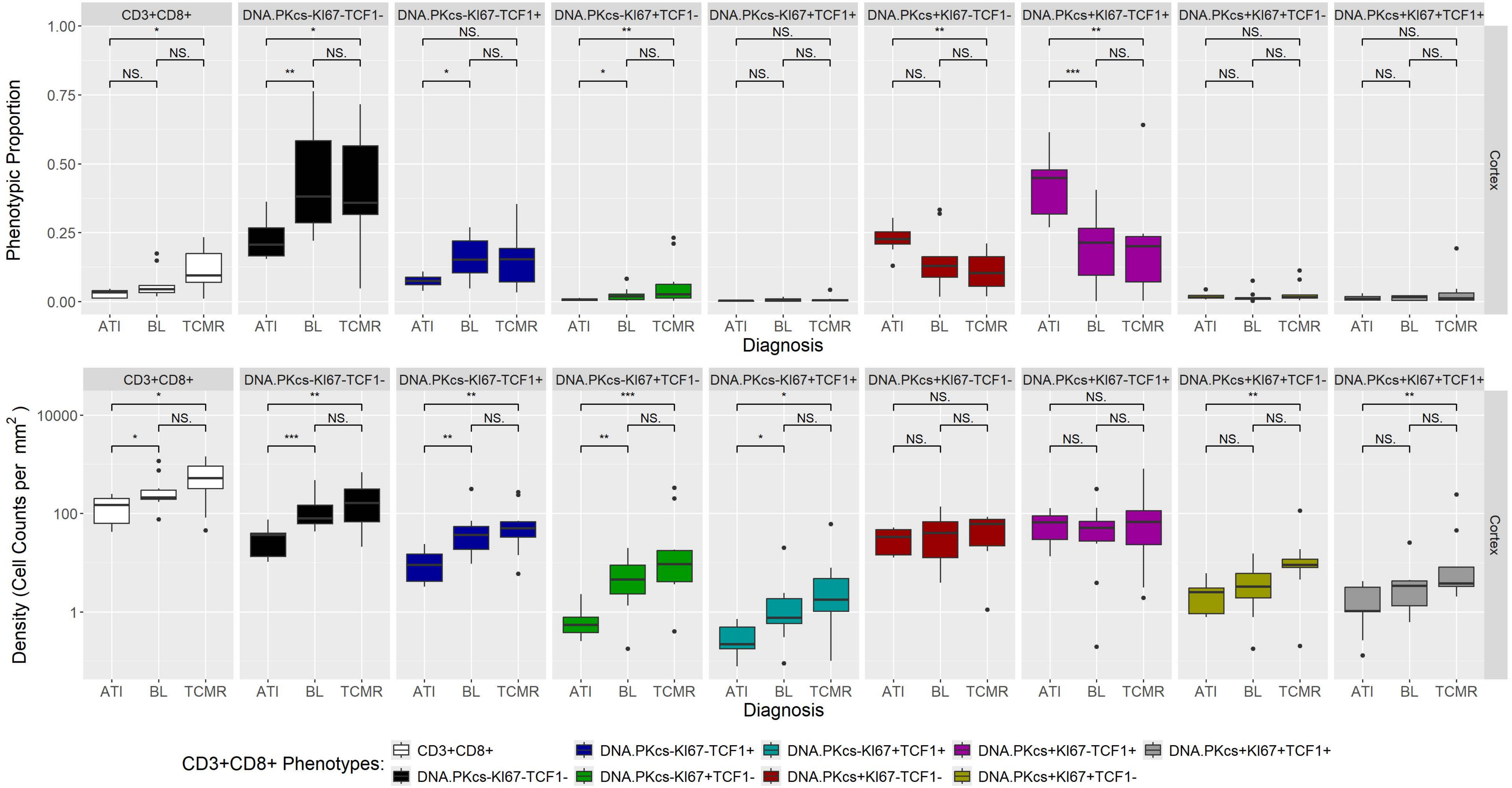
CONCLUSIONS

Intragraft CD8 T cells with different functional phenotypes can be localized using mIF. Intragraft CD8 T cells express the DNA repair enzyme, DNA-PKcs, suggesting a role for the DNA-repair complex in mature CD8 T cells located in peripheral tissues in the human. The inverse proportional relationship of DNA-PKcs expression to Ki67 & TCF1 expression within the intragraft CD8 T cell population in the progression of ATI to BL to TCMR may reflect immigration of CD8 T cells to the graft in early stages of inflammation (ATI and BL), with subsequent *in situ* expansion and maturation of the intragraft CD8 T cell population in TCMR.

Table 2. Mean Proportional Positivity of Each Phenotypic Marker in mIF Panel within the Total Cortical Cell Population Across Sample Groups

	Nuclear Density (cells/ mm ²)	CD3	CD8	Ki67	DNA-PKcs	TCF1	CC3	
ATI vs. TCMR	4749.92**	9.96***	2.90***	2.08***	65.66***	5.77***	13.10ns	* = p<0.05 ** = p<0.01 *** = p<0.001 ns = p>0.05
BL vs. ATI	5086.96ns	16.13**	6.75***	1.97ns	45.77***	6.20ns	9.48*	
TCMR vs. BL	5501.10ns	28.69***	12.79**	5.77***	43.80ns	14.06***	10.93ns	

Figure 2. Intragraft Cortical CD8 T cell Populations: Phenotypic Characteristics Across Sample Groups



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