CD206+ Resident Macrophages are a Candidate Biomarker for Renal Cystic Disease in Preclinical Models and Patients with ADPKD

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ABSTRACT

Although renal macrophages have been shown to contribute to cystic kidney disease in PKD animal models, it remains unclear if there is a specific macrophage subpopulation involved. Here we analyze changes in macrophage populations during renal maturation in association with cystogenesis rates in conditional *Pkd2* mutant mice. We demonstrate that a subset of resident macrophages with CD206 expression (CD206+ R2) are present during juvenile periods but decline during kidney maturation. This change correlates with the transition from rapid to slow cyst formation in the juvenile- and adult-induced *Pkd2* mutant models. We also assessed parallels between the CD206+ macrophage subset and disease severity in ADPKD patients and found that the number of CD206+ resident macrophage-like cells are also increased in kidneys from ADPKD patients, and furthermore, their urinary content correlated with the rate of renal functional loss in a ADPKD patient cohort. These data indicate that CD206+ resident macrophages correlate directly with cyst formation, and suggest that the CD206+ macrophage-like cells in the urine could serve as a non-invasive biomarker of cystic disease activity in ADPKD.

INTRODUCTION

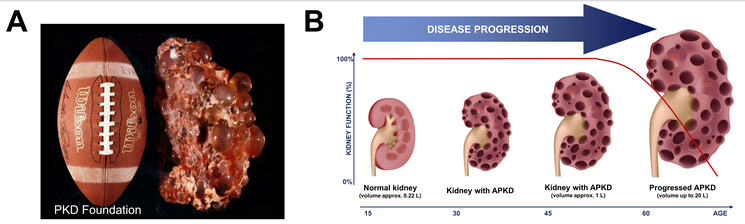

Figure 1. Disease Progression of ADPKD. (A) Human ADPKD Kidney. **(B)** Schematic of kidney volume and renal function decline over disease progression in ADPKD.

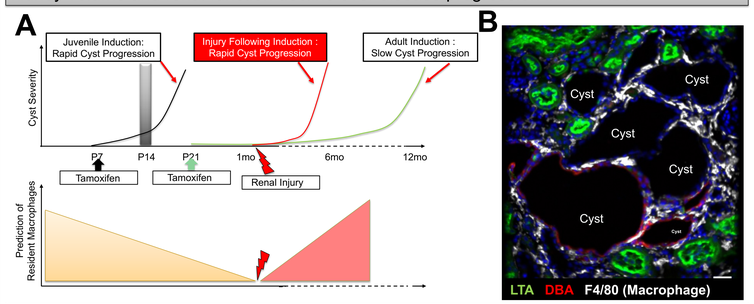

Figure 2. Renal Injury and Macrophages are Involved in Cyst Formation in Animal Models of ADPKD. (A) Timing of induced mutation determines the rate of cystogenesis in PKD mouse model **(B)** Macrophages accumulate around cysts in cystic kidneys.

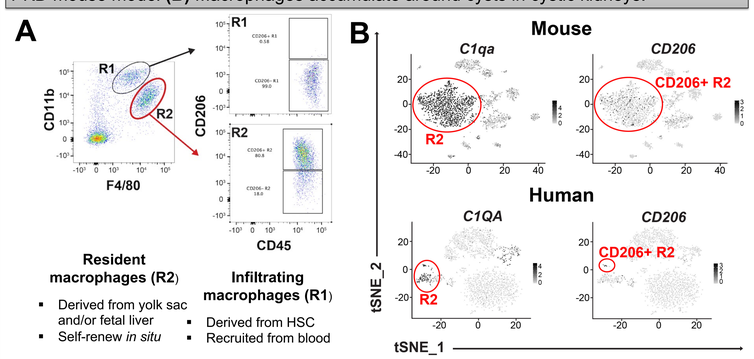

Figure 3. Infiltrating (R1) and Resident (R2) Macrophages in Kidney. (A) Gating strategy for R1 and R2 in kidney. **(B)** CD206+ resident macrophages are a conserved population of resident macrophages in kidney from mouse and human.

Resident Macrophages (R2) in Postnatal Kidney

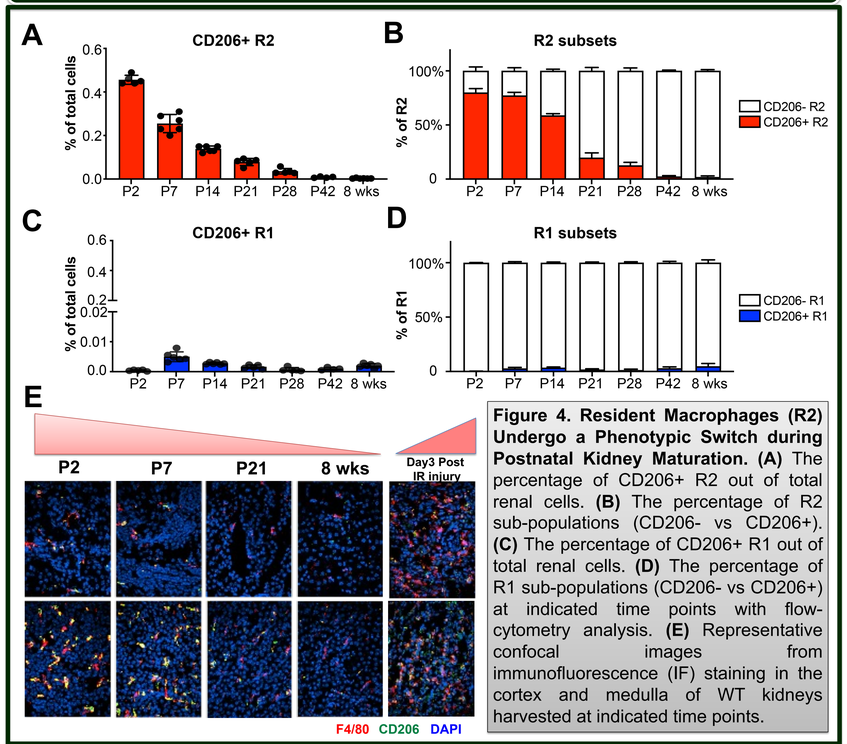

Figure 4. Resident Macrophages (R2) Undergo a Phenotypic Switch during Postnatal Kidney Maturation. (A) The percentage of CD206+ R2 out of total renal cells. **(B)** The percentage of R2 sub-populations (CD206- vs CD206+). **(C)** The percentage of CD206+ R1 out of total renal cells. **(D)** The percentage of R1 sub-populations (CD206- vs CD206+) at indicated time points with flow-cytometry analysis. **(E)** Representative confocal images from immunofluorescence (IF) staining in the cortex and medulla of WT kidneys harvested at indicated time points.

CD206+ R2 in Juvenile-induced Pkd2 Mutant Kidney

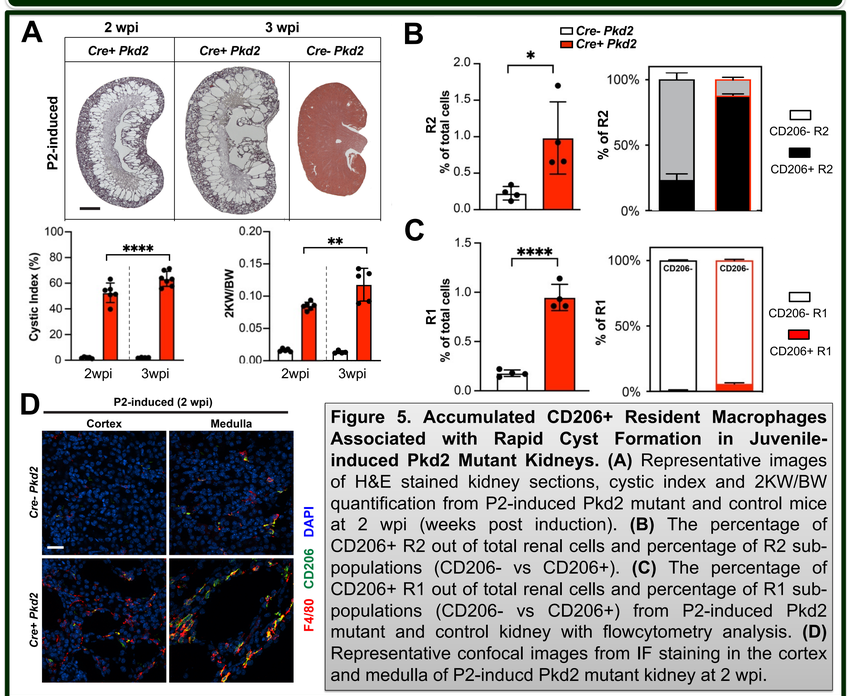

Figure 5. Accumulated CD206+ Resident Macrophages Associated with Rapid Cyst Formation in Juvenile-induced Pkd2 Mutant Kidneys. (A) Representative images of H&E stained kidney sections, cystic index and 2KW/BW quantification from P2-induced Pkd2 mutant and control mice at 2 wpi (weeks post induction). **(B)** The percentage of CD206+ R2 out of total renal cells and percentage of R2 sub-populations (CD206- vs CD206+). **(C)** The percentage of CD206+ R1 out of total renal cells and percentage of R1 sub-populations (CD206- vs CD206+) from P2-induced Pkd2 mutant and control kidney with flowcytometry analysis. **(D)** Representative confocal images from IF staining in the cortex and medulla of P2-inducd Pkd2 mutant kidney at 2 wpi.

CD206+ R2 in Adult-induced Pkd2 Mutant Kidney

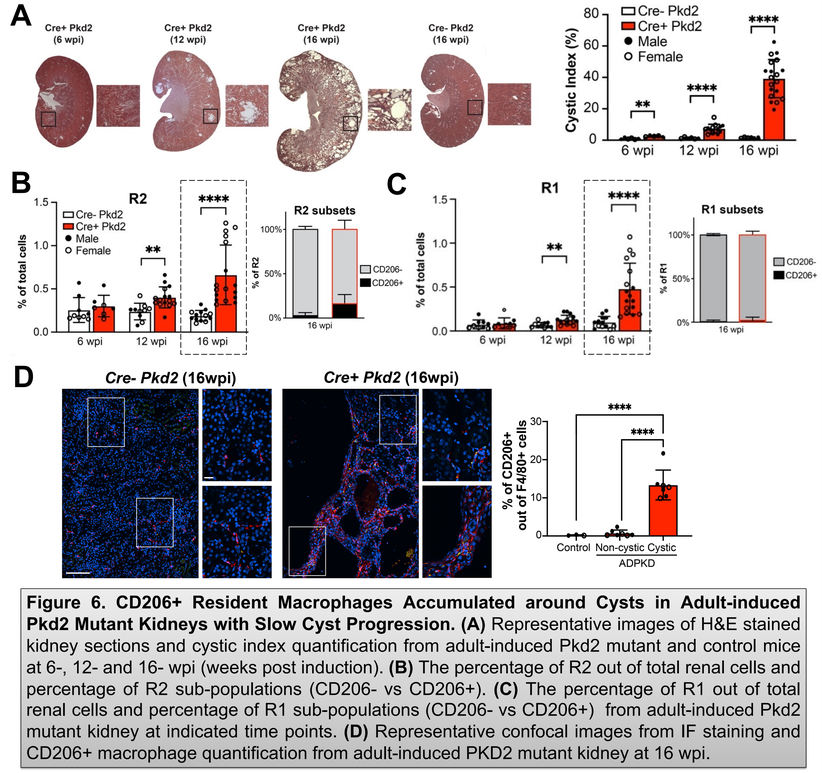

Figure 6. CD206+ Resident Macrophages Accumulated around Cysts in Adult-induced Pkd2 Mutant Kidneys with Slow Cyst Progression. (A) Representative images of H&E stained kidney sections and cystic index quantification from adult-induced Pkd2 mutant and control mice at 6-, 12- and 16- wpi (weeks post induction). **(B)** The percentage of R2 out of total renal cells and percentage of R2 sub-populations (CD206- vs CD206+). **(C)** The percentage of R1 out of total renal cells and percentage of R1 sub-populations (CD206- vs CD206+) from adult-induced Pkd2 mutant kidney at indicated time points. **(D)** Representative confocal images from IF staining and CD206+ macrophage quantification from adult-induced PKD2 mutant kidney at 16 wpi.

CD206+ Cells in Kidney and Urine from ADPKD Patients

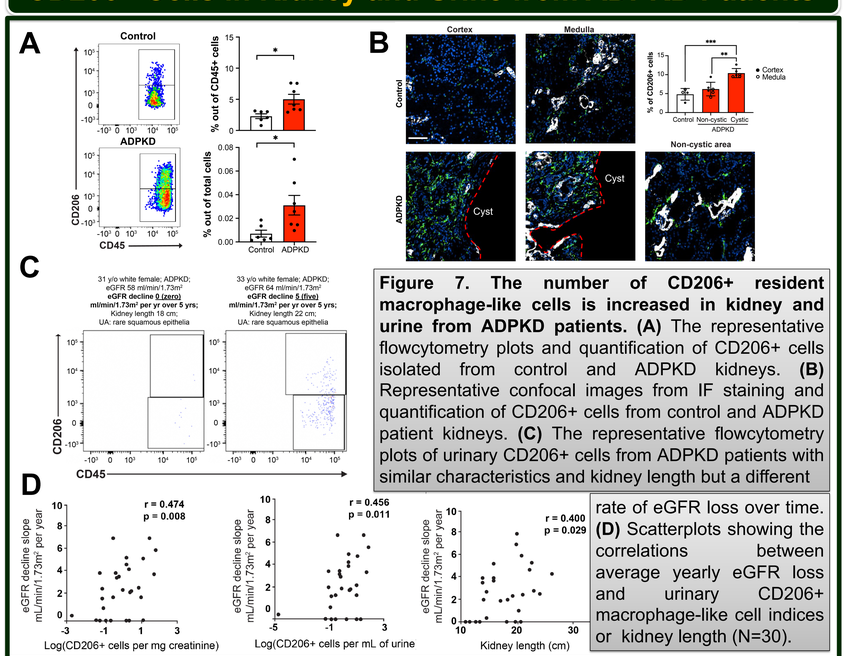

Figure 7. The number of CD206+ resident macrophage-like cells is increased in kidney and urine from ADPKD patients. (A) The representative flowcytometry plots and quantification of CD206+ cells isolated from control and ADPKD kidneys. **(B)** Representative confocal images from IF staining and quantification of CD206+ cells from control and ADPKD patient kidneys. **(C)** The representative flowcytometry plots of urinary CD206+ cells from ADPKD patients with similar characteristics and kidney length but a different rate of eGFR loss over time. **(D)** Scatterplots showing the correlations between average yearly eGFR loss and urinary CD206+ macrophage-like cell indices or kidney length (N=30).

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