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Live glomerular imaging in experimental crescentic glomerulonephritis reveals sequential glomerular infiltration of monocyte subsets with distinct effector functions

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BACKGROUND
Monocytes and their tissue descendants, macrophages, are known mediators of glomerular inflammation. However, heterogeneous populations of these cells exist. Non-classical (NC) monocytes crawl along resting endothelium, become pro-inflammatory upon detection of immune complexes (IC) and express high levels of FcγRIII(CD16). Classical monocytes are potent mediators of tissue inflammation, and a rich source of monocyte-derived macrophages. Nephrotoxic Nephritis (NTN) in the WKY rat (a susceptible strain) models glomerular inflammation caused by in situ IC formation and is a widely used, clinically-relevant model of crescentic glomerulonephritis(CrGN). However, the distinct roles of monocyte subsets and the dynamics of their glomerular recruitment are unknown.

METHODS
We developed a novel transgenic WKY-hCD68-GFP monocyte reporter rat strain. Intravital confocal microscopy, with in vivo antibody labelling, was performed in the hydronephrotic kidney of WKY-hCD68-GFP rats, permitting high-resolution live glomerular imaging and real-time, in vivo visualisation of monocyte subset recruitment and intravascular behaviour during NTN. Sorting of monocyte subsets and endothelial cells from glomerular isolates confirmed phenotype and effector functions during NTN. We explored the role of CD16 in IC detection, comparing Lewis and WKY-hCD68-GFP rats with NTN (CD16 genetic polymorphism mediates difference in NTN susceptibility).

RESULTS
Classical and NC monocytes were phenotyped as GFPposCD43loHIS48hi and GFPposCD43hiHIS48int respectively. RNA expression confirmed NC monocytes were CD16hiCX3CR1hiCD14loCCR2lo relative to classical monocytes i.e. homologous to cells in mice and humans.

Intravital glomerular imaging demonstrated highly dynamic interactions between intravascular monocytes and the glomerular endothelium. In the steady state, NC monocytes surveyed the endothelium via LFA-1 for prolonged periods. Classical monocytes had only transient endothelial interactions. During NTN, there were two waves of glomerular monocyte recruitment: LFA-1-dependent NC monocyte recruitment first, with subsequent retention of classical monocytes on day 6 NTN, coinciding with the onset of proteinuria. No transendothelial migration of these intravascular monocytes was observed.

There were subset-specific differences in the behavioural response to IC deposition during NTN: Increased recruitment of NC vs. increased retention of classical monocytes. Pro-inflammatory cytokines (IL-1β, TNFα) were upregulated in NC compared to classical monocytes and a unique chemokine axis was overexpressed by the endothelium and NC monocytes, which may orchestrate inflammatory myeloid cell recruitment and expression of damage mediators. RNA expression analysis indicated recruited glomerular cells were not M1- or M2-like macrophages, but predominantly inflammatory monocytes. Reduced classical monocyte

recruitment and absence of proteinuria during NTN in Lewis rats confirmed a role for FcγRIII(CD16) in mediating glomerular inflammation and damage.

CONCLUSIONS

We believe that development of this novel WKY-hCD68-GFP monocyte reporter rat strain and monocyte phenotyping panel has advanced the tools we have available to study mechanisms of glomerular inflammation in a clinically-relevant experimental model of CrGN.

Our data suggest intravascular monocytes, and not tissue macrophages, drive proliferative GN. NC monocytes monitor the glomerular endothelium through LFA-1 and detect IC through CD16, driving a pro-inflammatory phenotype that orchestrates the subsequent inflammatory response. This results in increased intravascular retention of classical monocytes which may drive glomerular damage, proteinuria and crescent formation. Targeting monocyte subsets may lead to effective treatments for GN, with fewer side effects.