Coaxing anti-inflammatory granulocytes to prevent ischemic kidney injury: a fine balance

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Neutrophils adhere to activated endothelium in peritubular capillaries and accumulate in the kidney interstitium less than 30 minutes after reperfusion following a period of ischemia. The traditional view is that these cells are pro-inflammatory and result in tubular injury. However, neutrophils are increasingly being characterised as a mixed population with pro- and anti-inflammatory effects. Unfortunately, the nomenclature and definition of these immunomodulatory cell types varies with the disease context, with diverse terms being used, such as “myeloid-derived suppressor cell (MDSC)”, “tumour-associated neutrophil”, “low density granulocyte” and “interphase neutrophil”. In the cancer field, these cells are generally felt to suppress the anti-tumour T-cell response and portend a poor prognosis, whereas in autoimmune disease such as SLE they may be pro-inflammatory (1). Given their effect on the immune response, their utility as a clinical tool in inflammatory disorders is a valid strand of investigation. However, dissecting out the context-specific effects of the various granulocyte subsets is an important first challenge.

In the current issue of the journal Yan et al propose that G-CSF induced MDSCs have a protective role in renal ischemia-reperfusion injury (IRI) in mice. This recapitulates data from previous rodent models of IRI, in which G-CSF attenuated renal injury and promoted anti-apoptotic pathways. However, the critical role of the G-CSF-induced suppressor cells was not appreciated in prior work. Flow cytometric and functional delineation of the MDSC populations, flawed as it is, is in line with current best practice in the manuscript by Yan et al. The authors used G-CSF to induce MDSCs and found that there was increased renal infiltration of these cells after IRI, that these facilitated renal recovery and ultimately attenuated renal fibrosis. They also found that administering G-CSF before IRI ameliorated acute tissue injury, renal apoptosis, and inflammation, all associated with induction of MDSCs. Of note, all experiments were performed in male mice, so it is not known whether there is a gender effect.

It is difficult to distinguish tissue based MDSCs from mature neutrophils and M2 macrophages; the authors recognise this and have taken extensive measures to try to account for it. To investigate the functional activity of the G-CSF induced MDSCs they found that the cells expressed increased levels of IL-10, Arginase-1 and reactive oxygen species. MDSCs were co-cultured with non-specifically activated splenic T cells and showed effective suppression of T cell function. Adoptive transfer of the purified MDSCs reduced the severity of renal IRI. Although G-CSF therapy alone was a good preventive measure for IRI, combining G-CSF treatment with an anti-Gr-1 antibody, aimed at depleting potentially damaging mature neutrophils, maintained MDSCs and augmented the benefit on IRI-mediated renal dysfunction over and above G-CSF treatment alone. Unfortunately, most of the therapeutic experiments involved pre-treatment with the agents, which would only have a therapeutic correlate in situations such as kidney transplantation or elective surgery in high risk populations.

G-CSF is used clinically in patients with congenital or acquired neutropenia, and for mobilisation of stem cells. It acts through its receptor on neutrophils and bone marrow precursor cells through the JAK/STAT, MAPK and PI3/Akt pathways. G-CSF is also implicated in neurogenesis and has been tested for protective effects in cerebral ischaemia. It has also shown beneficial effects in promoting coronary artery collateralization following ischaemia and is undergoing trials in the post myocardial infarction setting. Of note, therapeutic intervention with G-CSF is occasionally associated with

adverse events such as arteritis (2), Sweet's syndrome (3) and exacerbation of ANCA associated vasculitis and glomerulonephritis (4, 5).

The results of this study should be viewed in the light of a recent paper by Pegues et al examining the increase of g-MDSCs in response to C-reactive protein administration in IRI mice (6). This paper showed that direct depletion of g-MDSCs (using the same anti-Gr1 monoclonal antibody) reduced the albuminuria caused by renal IRI, suggesting that they play a deleterious role, in direct contrast with the current manuscript. Whereas Pegues et al used anti-GR1 antibody to deplete MDSCs, Yan et al suggest that the anti-GR1 antibody depletes the mature neutrophils, allowing the MDSC's to remain, even though both mature neutrophils and MDSC express Gr-1. Anti-Gr1 antibody mediated MDSC depletion, in the absence of inducible transgenic GR1+ cell knockouts or bone marrow transplant techniques, is thus fraught with difficulty (7, 8). The outcome will depend on the degree of relative depletion of mature murine neutrophils and MDSCs, which is difficult to determine accurately using flow cytometry. The authors should be commended by addressing this through additional use of pep-G3 and gemcitabine, although concerns about specificity inevitably remain.

The significance of this paper is that it suggests a positive therapeutic role for G-CSF induced MDSCs in IRI in mice and a potential useful therapeutic for human IRI. However, translation of the combined G-CSF treatment with anti-Gr-1 may prove difficult as there is no known direct human homolog of Gr-1. Human CD177, the binding partner of surface expressed proteinase-3 (of great interest in the ANCA vasculitis field), is structurally related to this molecule and may be a potential surrogate human target (9). Alternatively, other agents targeting mature neutrophil recruitment may also be testable substitutes, such as CXCR2 antagonists or C5a receptor antagonists, both currently in human clinical trials.

The use of human G-CSF in a murine study also limits translational potential. There is only 73% amino acid sequence homology between human and mouse G-CSF. The authors used a markedly elevated dose to overcome this species difference, although this does raise some concerns about unmeasured off-target effects from these supra-normal doses, and how this effect could be translated to the clinical arena. In addition, it is known that renal ischaemia/hypoxia itself promotes human renal tubular cell expression of G-CSF, which at physiological concentrations may already be mediating some reno-protective effects, so the degree of benefit attainable at appropriate pharmacological concentrations is uncertain.

Since G-CSF therapy is already clinically approved and undergoing human trials in other forms of ischaemic injury, it could be readily tested in renal IRI in the context of transplantation or in high risk subjects undergoing vascular surgery. However, to progress this, it would be essential to first develop mechanisms to define the various neutrophil subsets in humans. This would allow tailoring of therapy to ensure that the correct balance of pro and anti-inflammatory neutrophils is entrained. The exact human correlate of the MDSCs studied by Yan et al remains unclear. Cross-disciplinary agreement on characterisation of low-density granulocytes and MDSCs is thus urgently needed.

References:

1. Rahman S, Sagar D, Hanna RN, Lightfoot YL, Mistry P, Smith CK, et al. Low-density granulocytes activate T cells and demonstrate a non-suppressive role in systemic lupus erythematosus. Ann Rheum Dis. 2019;78(7):957-66.
2. Sasaki K, Miyauchi M, Ogura M, Shimura-Nukina A, Toyama K, Nakazaki K, et al. Arteritis after administration of granulocyte colony-stimulating factor: a case series. Int J Hematol. 2019;110(3):370-4.
3. Paydas S, Sahin B, Seyrek E, Soylu M, Gonlusen G, Acar A, et al. Sweet's syndrome associated with G-CSF. British journal of haematology. 1993;85(1):191-2.
4. Freeley SJ, Coughlan AM, Popat RJ, Dunn-Walters DK, Robson MG. Granulocyte colony stimulating factor exacerbates antineutrophil cytoplasmic antibody vasculitis. Ann Rheum Dis. 2012.
5. Iking-Konert C, Ostendorf B, Foede M, Fischer-Betz R, Jung G, Haensch MG, et al. Granulocyte colony-stimulating factor induces disease flare in patients with antineutrophil cytoplasmic antibody-associated vasculitis. J Rheumatol. 2004;31(8):1655-8.
6. Pegues MA, McWilliams IL, Szalai AJ. C-reactive protein exacerbates renal ischemia-reperfusion injury: are myeloid-derived suppressor cells to blame? Am J Physiol Renal Physiol. 2016;311(1):F176-81.
7. Wang XY, Yi H, Li J. Response to: 'Issues with anti-Gr1 antibody-mediated myeloid-derived suppressor cell depletion' by Xing et al. Ann Rheum Dis. 2016;75(8):e50.
8. Xing YF, Zhou YQ, Ma GW, Feng DY, Cai XR, Li X. Issues with anti-Gr1 antibody-mediated myeloid-derived suppressor cell depletion. Ann Rheum Dis. 2016;75(8):e49.
9. Hickey MJ. Has Ly6G finally found a job? Blood. 2012;120(7):1352-3.