

Response to: 'Issues with anti-Gr1 antibody-mediated myeloid-derived suppressor cell depletion' by Xing *et al*

Myeloid-derived suppressor cell (MDSC) is a heterogeneous myeloid cell population that can broadly be characterised as CD11b⁺Gr-1⁺ cells in mice. MDSC can be further classified into two main cell subsets, granulocytic MDSC (G-MDSC, CD11b⁺Ly6C^{low}Ly6G⁺) and monocytic MDSC (M-MDSC, CD11b⁺Ly6C^{high}Ly6G⁻).¹ To address the role of MDSC in disease settings (eg, cancer and autoimmune disorders) and to evaluate the potential therapeutic benefits of targeting MDSC in preclinical models, different strategies, including anti-Gr-1 antibodies²⁻⁵ and pharmacological inhibitors (eg, gemcitabine and 5-fluorouracil),^{6,7} have been widely used to selectively remove mouse MDSC. The use of these reagents in combination with appropriate experimental systems has advanced significantly our current understanding of these myeloid cells in multiple physiological and pathological processes. Xing *et al*⁸ raised an issue with the use of anti-Gr-1 (RB6-8C5) for depletion of MDSC, which indeed has long been recognised by the research community. Anti-Gr-1 antibodies can bind to two Ly6 superfamily molecules, Ly6G and Ly6C, which are preferentially expressed on the granulocytes and monocytes, respectively. The differential expression levels of Gr-1 on G-MDSC and M-MDSC may result in different sensitivities to cross-linking of or depletion with anti-Gr-1 antibodies. In some cases, administration of anti-Gr-1 antibodies may cause compensatory rebound of Gr-1⁺ cells, and therefore multiple treatments are necessary to maintain depletion efficiency *in vivo*. While the capacity of anti-Gr-1 antibodies to deplete peripheral MDSC in spleen or in the circulation system has been well documented, their effect on tissue MDSC or those in the diseased sites remain less clear. The discrepancies pointed out by Xing *et al* may have been attributed to different models or regimens of antibody administration used in these studies. In the study by Ma *et al*⁹ that involved depletion of hepatic MDSC, anti-Gr-1 antibodies were only given once. Considering that depletion efficiency can be potentially altered by multiple factors, such as the accessibility of antibodies and the time needed for cell turnover in certain tissues (eg, liver), it is possible that a single treatment with anti-Gr-1 antibodies may not sufficiently cause significant reduction of MDSC in those tissues. Furthermore, relying on antibody depletion alone may not be adequate for defining the role of MDSC in pathogenesis of diseases, including inflammatory arthritis. Other complementary approaches, such as pharmacological depletion of MDSC¹⁰ and adoptive cell transfer,^{10,11} as we recently described, must be used to stringently and comprehensively examine the contribution of MDSC or its subsets to immune evasion, inflammatory response, immune homeostasis or disease pathogenesis. Our finding of MDSC in enhancing inflammatory Th17 response in collagen-induced mouse arthritis model was also supported by an independent study by Zhang *et al*,¹² in which anti-Gr-1 antibodies were similarly used as an experimental approach to demonstrate a pathogenic effect of MDSC. Therefore, it is likely that anti-Gr-1 antibodies may not completely remove MDSC in arthritic mice, its impact on the pathogenic progression of arthritis in mice is evident.

MDSC was originally described as immunosuppressive myeloid cells involved in cancer evasion. However, emerging evidence, including our recent data, revealed that MDSC induced differentiation of proinflammatory Th17 cells in autoimmune disease models, for example, experimental autoimmune

encephalomyelitis,¹⁰ collagen-induced arthritis¹¹ or the tumour-bearing host.¹³ These apparently controversial observations were certainly not caused by the inappropriate use of reagents. On the contrary, we believe that these new findings highlight the pleiotropic functions of MDSC in distinct physiological or pathological states that were not previously appreciated. The elegant study by Kumar *et al*³ revealed transcriptional factor signal transducer and activator of transcription 3 (STAT3)-mediated regulation of the fate of myeloid cells in tumour-bearing hosts, especially in hypoxic tumour environment. Anti-Gr-1 antibodies in this study were used to achieve short-time depletion of MDSC in blood and spleen, which was designed to test whether the observed decrease in tumour-associated macrophage (TAM) in STAT3C (ie, constitutive STAT3 activation in myeloid cells) mice was attributed to increased recruitment of MDSCs. Anti-Gr-1 antibodies depleted only G-MDSC, but had little effect on M-MDSC or TAM in tumours. Importantly, inhibition of tumour growth required combined MDSC depletion with anti-Gr-1 antibodies and STAT3 activation in myeloid cells, suggesting that G-MDSC and M-MDSC-TAM axis contribute to tumour progression and can compensate for each other loss. Considering the multiple active tumour-promoting pathways present in tumour-bearing host, it is not surprising that elimination of only one cell populations, for example, MDSC, is insufficient to achieve an effective antitumour response.³ These findings emphasise the complexity of tumour-promoting myeloid cell network that operates in the tumour environment, and indicate that understanding of action of MDSC in the host responses and disease progression is far from complete.

MDSC research is a fast-moving and exciting field with potential major impact on public health. Despite the limitation of experimental reagents, such as anti-Gr-1 antibodies, for depleting MDSC or its subsets in some tissues, we believe that they will continue to serve as a useful research tool, when rationally combined with other approaches, to help dissecting the pathophysiological roles of MDSC. The multifaceted and/or context-dependent functions of these highly plastic myeloid cells in different diseases are beginning to be appreciated.¹⁴ Although MDSC represents a major cellular component involved in immune evasion in tumour-bearing host, caution must be used when one predicts or expects an enhanced antitumour response on depletion of such a cell population with anti-Gr-1 antibodies or other MDSC-depleting agents. It is increasingly clear that tumour progression and immune evasion are mediated by multiple known or yet unknown cellular and molecular mechanisms. With new reagents being developed to more efficiently either deplete MDSC¹⁵ or modify functions of these cells,¹⁶ the researchers are poised to identify and define the diverse activities of MDSCs beyond the already established immunosuppressive feature, which is expected to lead to novel biological or pharmacological therapeutics targeting MDSCs for benefiting patients with cancer and autoimmune diseases.

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Funding National Cancer Institute (grant numbers: CA175033, CA154708).

Competing interests None declared.

Provenance and peer review Commissioned; internally peer reviewed.



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To cite Wang X-Y, Yi H, Li J. *Ann Rheum Dis* 2016;**75**:e50.

Received 9 May 2016

Accepted 14 May 2016

Published Online First 2 June 2016



► <http://dx.doi.org/10.1136/annrheumdis-2016-209786>

Ann Rheum Dis 2016;**75**:e50. doi:10.1136/annrheumdis-2016-209848

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