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Authors' Reply

Authors' reply to 'Granulocytic myeloid-derived suppressor cells in peripheral blood of patients with cutaneous melanoma'

We are grateful to Dr Franklin and Dr Schilling for their interest in our recent article (1) and we will try to respond to their constructive critical review.

In regard to Dr Franklin's and Dr Schilling's emphasis that the $CD10^{-}CD15^{+}CD14^{-}HLA\text{-}DR^{-/low}CD33^{low}CD11b^{+}CD45^{low}CD16^{low}Lin^{-}$ panel is '… a widely used panel for identifying grMDSC in PBMC after density centrifugation', we would like to underline that we initially conducted a pilot study showing that cells with the above-mentioned phenotype copurified with the PBMC on density gradients (Supplementary Figure 1, available at *International Immunology* Online). The reasons why we decided to continue the study with lysed peripheral blood samples were listed in the article (1). We agree that the activated neutrophils can change their buoyancy and copurify on a density gradient, but these activated cells are still $CD10^{+}$ and the CD10 expression could even be up-regulated in activated mature neutrophils (2).

Regarding the remark on the separation of the grMDSC from eosinophils, besides being $CD10^{-}$, eosinophils are also $CD16^{-}$, $CD45^{bright}$ and high on side scatter (SSc) (3). Our targeted population showed low, but positive CD16 expression, low CD45 expression and a low SSc position (1). Additionally, no eosinophilia was recognized in differential blood counts in our patients with >10% of the presumed grMDSC population. Accordingly, we disagree with Dr Franklin's and Dr Schilling's assertion that we have distinguished eosinophils and grMDSC on the basis solely of low or absent CD16 expression.

We agree that antigen expression changes with aging; however, both the grMDSC frequencies and the CD16 expression intensity did not correlate with the patient's age in our study. Subsequently, we detected CD66b on our grMDSC, which could separate them from immature myeloid cells, according to the results of Dumitru's group, cited by Dr Franklin and Dr Schilling.

We agree that the functional assay is the most reliable evidence for immunosuppressive activity of certain cell populations; thus, we titled our manuscript as 'A subpopulation that may correspond to ...' (1). On the other side, the classical suppressive assay can exclude neither the influence of activated healthy granulocytes, which are also capable of T-cell suppression (4), nor the possibility of MDSC activation during long-time cultivation with T cells (5). The suppressive assay is certainly superior in the situation of testing MDSC from the site of inflammation or the tumor-growing site, because those cells possess the immediate capacity to down-regulate T-cell proliferation *in vitro* (5). Instead of using the classical suppressive assay, we identified our grMDSC by phenotype (1) and investigated possible *in vivo* grMDSC suppressive outcomes related to arginine depletion, oxidative stress and immune response skewing (Supplementary Figure 2, available at *International Immunology* Online).

At the end, we consider as a weakness of our study the fact that we have not performed repeated measurements of the grMDSC frequencies during the follow-up period.

Supplementary data

Supplementary data are available at *International Immunology* Online.

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