Reviewer’s report

**Title:** Matrix metalloproteases as maestros for the dual role of LPS- and IL-10-stimulated macrophages in cancer cell behaviour

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**Reviewer:** Nor Eddine Sounni

**Reviewer’s report:**

In this work, authors investigated the role of LPS versus IL10 activated macrophages on gastric and colorectal cancer cell invasion, migration and angiogenesis. Results show that macrophages modulate cancer cell invasion, motility, cancer cells induced angiogenesis. These phenotypes are more efficient with IL-10-stimulated macrophages than LPS. EGFR is essential for both macrophage populations-mediated invasion. However, no difference was seen in downstream signaling, including, Src, ERK1/2, Akt and p38 phosphorylation. Mechanistically, authors claim higher proteolytic activities of MMP2 and MMP9 in IL-10-stimulated macrophages, which correlate with their ability to stimulate cancer cell invasion and angiogenesis. Conclusions drawn from the study are not supported by the data presented for the MMP activation profile.

**Major comments:**

MMP2 and MMP9 production was assessed by gelatin zymography analysis of CM from cancer cells activated macrophages, or cancer cells incubated with CM from LPS or IL10 stimulated macrophages. However, it is very hard to believe author’s claims, as no difference is seen between LPS and IL-10 activated macrophages for pro-MMP9 or pro-MMP2 production levels. Authors are able to see differences in MMP2, -9 activities based on their evaluation of densitometry scanning of the active form of enzyme to its pro-form. Again, it impossible to see any increase in the active form of MMP2 of MMP9 and no active form is seen on the illustrated zymogramme. Gelatin zymography is highly sensitive technique; it allows the detection of 100 pg of active or inactive enzyme in medium or biological fluids. If no band was detected for the active form of MMP2 or MMP9, this means that biological effects of macrophages CM are not dependent on MMP’s proteolytic activities.

**Minor comments**

1) EGFR is shown at 150 kDa, it should be at 175kDa
2) MMP2 and MMP9 expression should be confirmed by WB or ELISA.
3) CM from non stimulated macrophages is missing in the zymogramme. All conditions should be run on the same gel.
4) For molecular weight of active forms of MMP2 and MMP9, authors can use a positive control of cancer cells stimulated by PMA or Concavalin-A. As
suggestion, authors can use HT1080 for MMP-2 and -9 or other cells such as, MDA-MB231 cell for MMP2 or MCF7 for MMP9.

5) In figure 1D, expression of CD163 is similar between mac and mac IL10 condition. In result section, authors’ state increased CD163 in IL10 stimulated macrophages when compared to LPS condition; however, this comparison should be done between IL10 and control condition.

**Level of interest:** An article of limited interest

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

Reviewer declares no conflict of interest