SCIENTIFIC REPORT

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STSM Subject: miR-125b drives P2X7r-mediated activation of NLRP3 inflammasome in microglia

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RATIONALE

Microglia are the CNS resident macrophages that can be compared to hematopoietic macrophages for shape and function and, as macrophages, they can be polarized toward two different general states: M1/pro-inflammatory or M2/anti-inflammatory *(Franco R et al, Prog Neurobiol 2015).* However, it is now clear that hematopoyetic macrophages and microglia have different progenitors, as a significant proportion of microglia cells arises from the yolk sac and populates the neuroepithelium in early (before E10.5) embryogenesis *(Ginoux et al. 2010).* This different origin should thus explain the peculiar responses of microglia to innate immune stimuli when compared to hematopoietic macrophages *(Xiao et al. 2013).*

In our lab, we have demonstrated that P2X7r-mediated microglia activation is controlled by miR-125b *(Parisi C et al. 2013)* targeting the NF-kB inhibitor A20 *(Parisi C et al. 2016)*. In particular, we have proved that A20 is a target of miR-125b and that miR-125b inhibition is able to inhibit p65/NF-kB pathway in activated microglia by restoring A20 levels, with consequent decrease of M1 pro-inflammatory markers *(Parisi C et al. 2016).* Moreover, miR-125b is mostly expressed in macrophages within the peripheral immune cells and its over-expression in bone marrow derived macrophages (BMDM) potentiates M1 activation *(Chauduri AA et al 2011).* M1 phenotype in both macrophages and microglia is characterized by NLRP3 inflammasome activity that is strongly induced by ATP acting through P2X7r *(Mariathasan S et al. 2006; Hanamsagar R et al, 2011).* A recent work has demonstrated that A20 restricts NLRP3 inflammasome activation by ATP in macrophages, through direct interaction with inflammasome proteins (*Duong BH et al. 2015*). Surprisingly, in macrophages polarized towards the M2 phenotype, P2X7r is uncoupled from NLRP3-inflammasome activation and IL-1beta production *(Pelegrin P et al, EMBO J 2009)*.

AIMS

The general aim of the proposed research was the characterization of P2X7r-mediated NLRP3-inflammasome activity and miR-125b/A20 expression in primary microglia over a macrophage dynamic polarity gradient *(Pelegrin P et al EMBO J 2009).*

In detail, the research was focused on:

- Microglia vs BMDM polarization properties and P2X7r-mediated inflammasome activity when exposed to the dynamic polarity gradient;
- MiR-125b/A20 variations in BMDM and microglia during the polarization dynamics and after P2X7r activation.

RESULTS

Figure 1. A20 is differentially modulated during the polarity gradient in BMDM, with major expression found in M1 phases

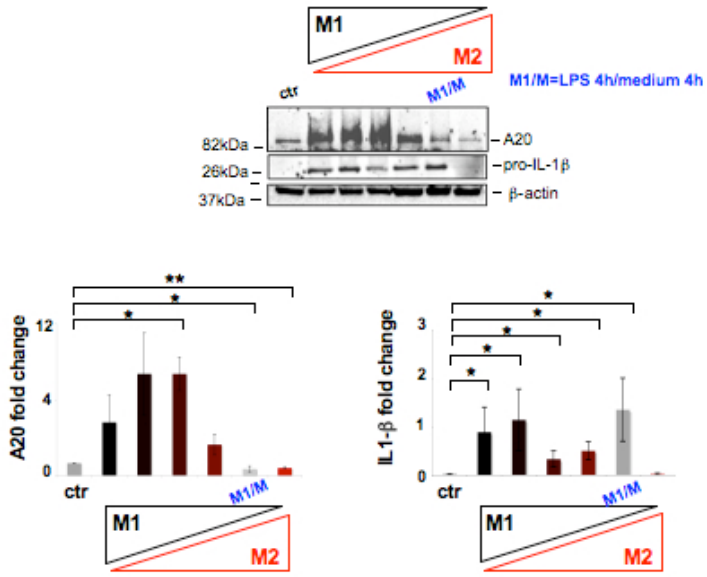


Figure 2. A20 is differentially modulated by extracellular ATP during the polarity gradient in BMDM, with ATP inhibiting A20 expression in M1 phases, and stimulating it in M2 phases

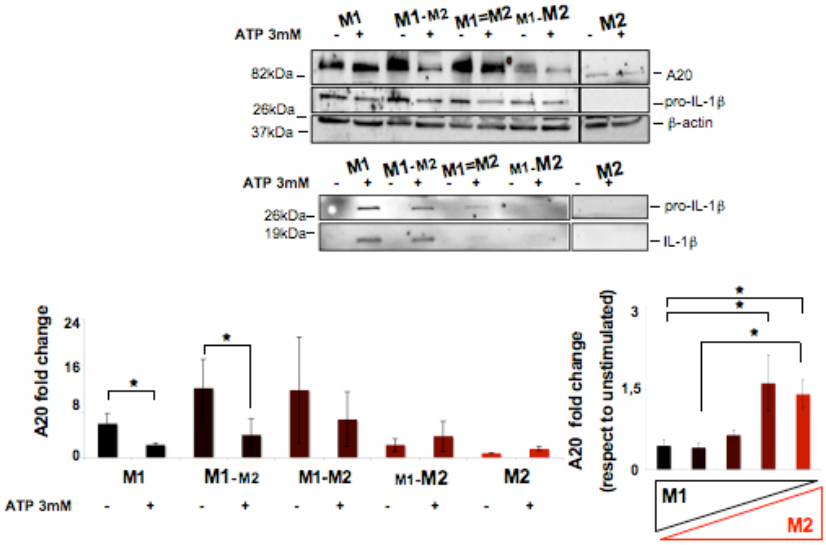


Figure 3. ATP-mediated IL-1β production is reduced during the polarity gradient in BMDM, with ATP always stimulating its expression

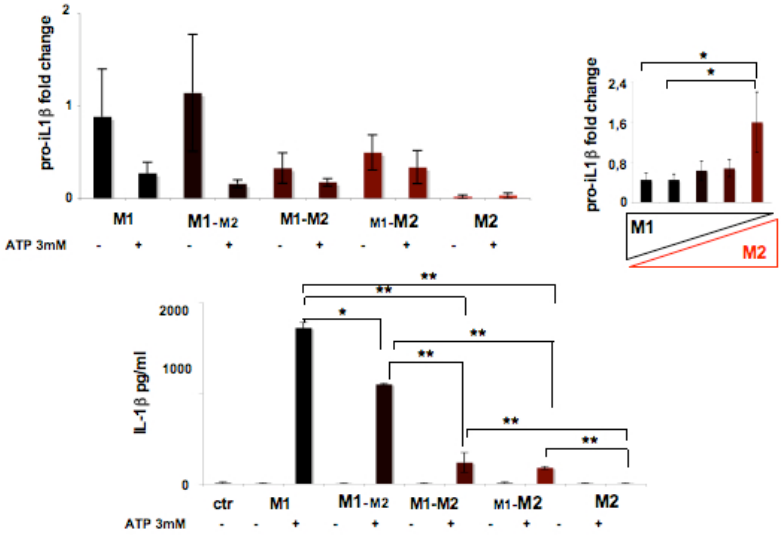


Figure 4. ATP-induced cell death is reduced in BMDM during the transition from M1 to M2 phases

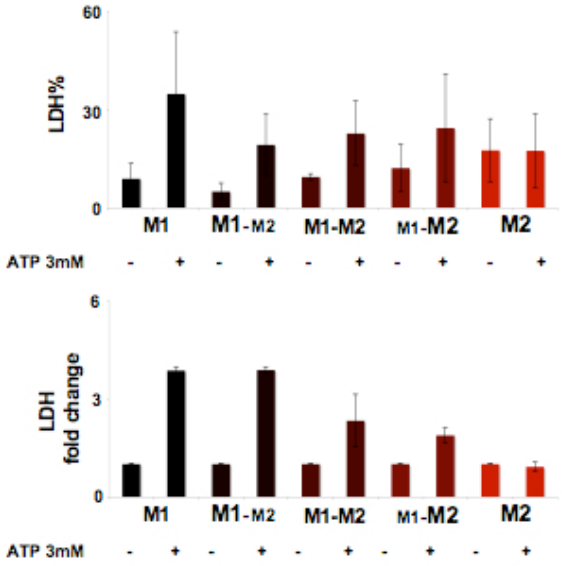


Figure 5. **A20 is both induced and inhibited by ATP in microglia during the polarity gradient**

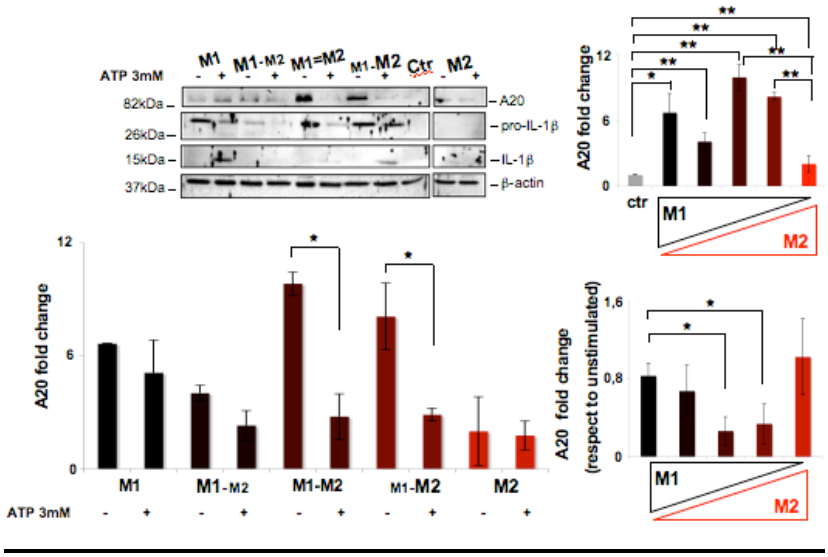


Figure 6. **Pro-IL1β synthesis and processing are both induced and inhibited by ATP in microglia during the polarity gradient**

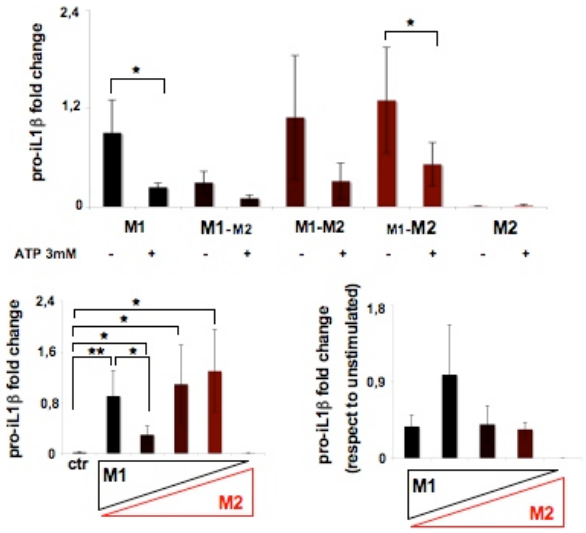


Figure 7. ATP-induced IL-1β production and cell death are both induced and inhibited in microglia during the polarity gradient

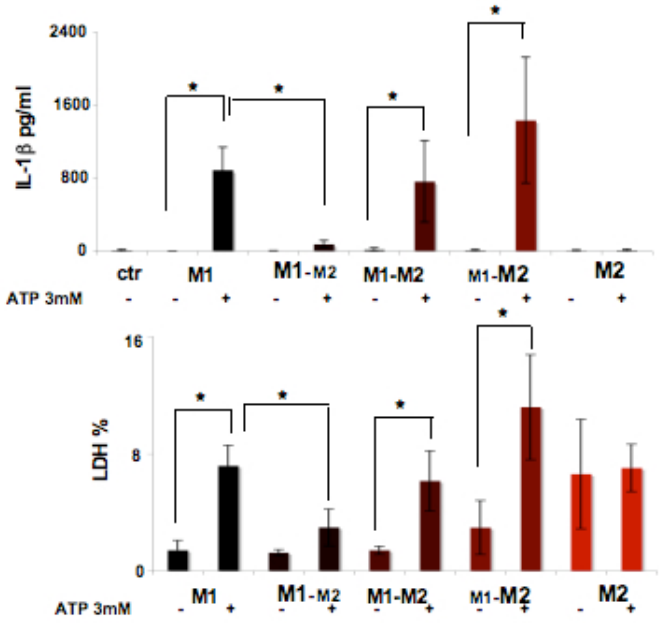


Figure 8. MiR-125b is modulated during the polarity gradient in BMDM, with ATP stimulating its expression in M1 phases

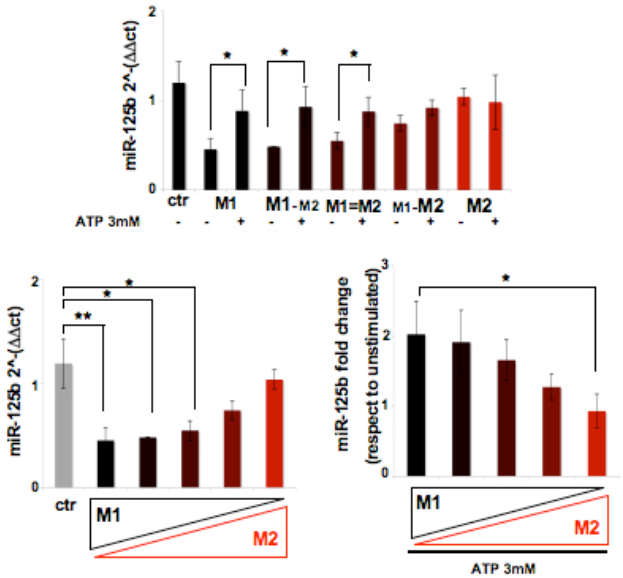


Figure 9. **MiR-125b is modulated during the polarity gradient in microglia, with ATP stimulating its expression only in the first M1 phase, i.e. in the absence of IL-4.**

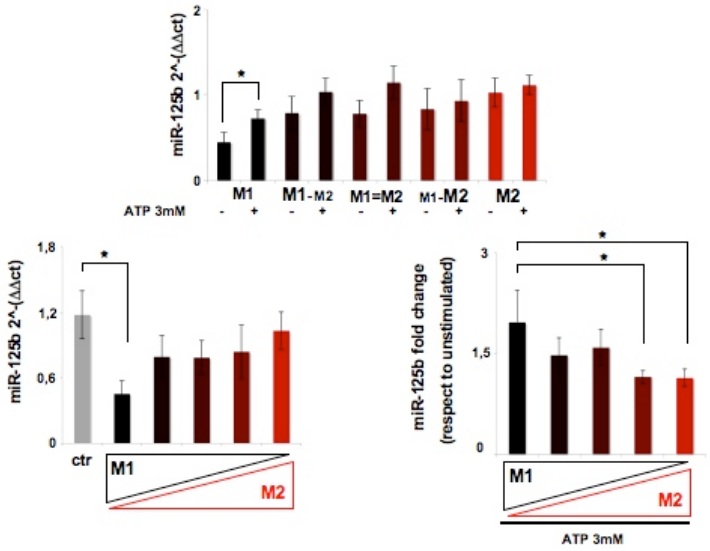


Figure 10. **Markers for M1 (TNFα) and M2 (MRC-1) polarization reveal that microglia do not polarize as macrophages**

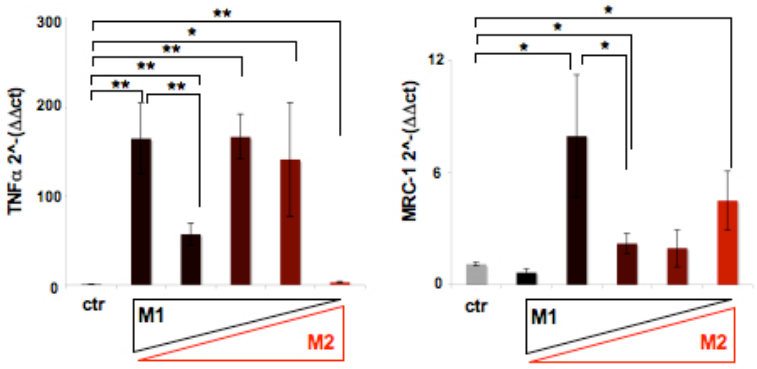
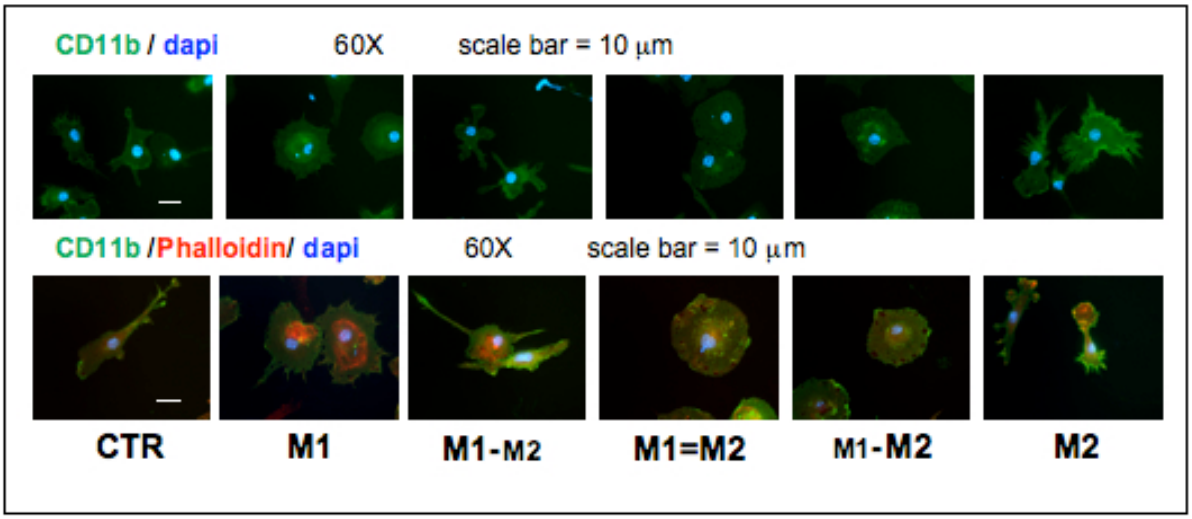


Figure 11. Microglia morphology confirms that they do not polarize as macrophages



Conclusions

The general aim of the proposed research was to characterize miR-125b/A20 expression in primary microglia over a macrophage dynamic polarity gradient. By performing comparative Elisa, western blot, quantitative PCR, metabolic and immunofluorescence analysis, we have demonstrated that microglia do not polarize as macrophages, when sequentially exposed to IL-4 /LPS and vice versa, while BMDM mostly behave as peritoneal macrophages.

In particular, we have found that the response to ATP of microglia is quite similar to that of BMDM when analyzed in strict M1 (LPS challenge for 4 hours) or M2 (IL-4 challenge for 4 hours) phases, while a biphasic response is instead obtained in M1/M2 intermediate phases only in microglia. This trend regards Il-1β, TNFα and MRC-1 production, sensitivity to toxic extracellular ATP concentration (3mM) and morphological transition of the cells.

Differently from BMDM where LPS and IL-4 mutually sustain a linear M1/M2 inversely proportional gradient, we have observed that in microglia LPS (before IL-4) completely prevents the M2 phases and potentiates the response to extracellular ATP, thus priming microglia toward an M1 inflammatory phenotype. Conversely, IL-4 (before LPS) seems to completely impede the microglia M1 response, and to enhance the M2 response, thus priming microglia toward an anti-inflammatory M2 phenotype.

Within this picture, miR125b and A20 behave consequently in microglia and in microphages distinguished by a different progression into M1/M2 phases. We have indeed found that miR125b expression is more activated by ATP in initial M1 than late M2 phases in both microglia and BMDM, while A20 levels are inhibited by ATP in M1 phases in macrophages and again inhibited in a transition M2 phase in microglia.