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Inflammasome Activation in M2 Macrophage Restrain the Immune Suppressive Function

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Background

Macrophage is an important component in tumor microenvironment (TME) and plays multiple roles in tumor initiation, progression and metastases. In response to various stimuli within TME, macrophage exhibits high level of functional heterogeneity. There are two distinct groups of macrophages: M1 macrophage exhibits pro-inflammatory phenotype with high levels of TNF-α, IL-6, and IL-1β, while M2 macrophage displays immune suppressive phenotype with high levels of anti-inflammatory cytokines such as IL-10 and TGF-β. In response to the M2 cytokines, myeloid cells within the TME further acquire higher expression of PD-L1 and thus inactivate T cells. M2 cytokines can also directly inhibit T cell activation. As a result, re-polarizing M2 macrophages becomes a key concept for cancer immunotherapy. The NLRP3 inflammasome is acquired by macrophages to fight against endogenous danger signals. Macrophage NLRP3 activation has been observed in several tumor models, but the function of NLRP3 on macrophage polarity remains controversial. Inflammasome activation with IL-1β/IL-18 secretion was reported to promote M1 polarization. However, NLRP3 activation was also reported to promote M2 polarity through up-regulation of IL4 in asthma model. Here, we have established an in vitro human macrophage NLRP3 activation system, coupled with M2 macrophage polarization assay, to dissect the role of NLRP3 in macrophage phenotype. Our results indicate that NLRP3 activation restrained M2 phenotype and further enhanced T cell activation in an M2/T cell co-culture system.

Methods

Human macrophages were generated from primary human monocytes, which were isolated from human PBMC using CD14+ monocyte isolation kit. For polarization to the M1 phenotype, CD14+ peripheral blood mononuclear cells (PBMCs) were given GM-CSF for 5 days, and cells were stimulated with GM-CSF in concert with LPS and IFN-γ for an additional 24 hours on Day 5 . For M2 polarization, cells were treated with M-CSF for 5 days, then cells were stimulated with M-CSF in concert with IL-4 and IL-13 for an additional 24 hours.

M2 cells were treated with LPS for 24h, followed by ATP for another 24h. Cells were collected to analyze the surface markers expression or co-culture with CellTrace labeled T cells.

Results

Human macrophages M1 expresses CD86 and release pro-inflammatory cytokines, while M2 has a high level of CD163 and produces anti-inflammatory cytokines.

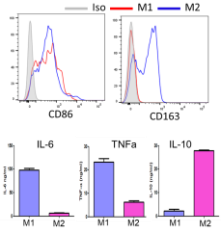

Figure 1 Human M1 and M2 macrophage polarization and characterization

LPS couple with ATP or Nigericin induces inflammasome activation and promote IL-1β production in human M1 and M2 macrophage.

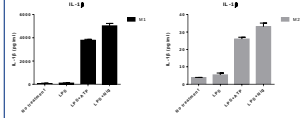

Figure 2 Inflammasome activation and IL-1β production in human macrophages M1 and M2.

LPS coulple with ATP decreases CD163 expression in M2 macrophage. Caspase-1 cleavage and IL-1β production could be detected in M2 after LPS/ATP stimulation.

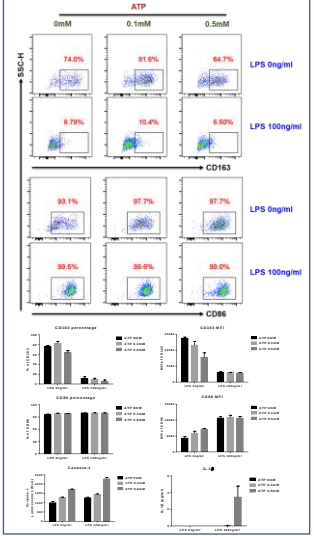

Figure 3 CD163, CD86 expression, caspase-1 cleavage and IL-1β production in LPS/ATP treated M2.

LPS/ATP treated M2 promotes T cell proliferation and activation.

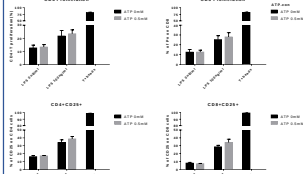

Figure 4 T cell proliferation and CD25 expression on T cells were analyzed after 3 days of M2 and T co-culture.

Conclusions

- LPS couple with ATP or Nigericin induces inflammasome activation and promote IL-1β production in M1 and M2 macrophage.
- Inflammasome activation in M2 could reduce CD163 expression.
- Inflammasome activated M2 could promote T cell proliferation and activation compare to naïve M2, suggest inflammasome activation may potentially reverse anti-inflammatory phenotype of macrophage.

References

- Lin Y., et al. Tumor-associated macrophages in tumor metastasis: biological roles and clinical therapeutic applications. J Hematol Oncol. 2019; 12: 76.
- Liu Y., et al. NLRP3 regulates macrophage M2 polarization through up-regulation of IL-4 in asthma. Biochem J. 2018;475(12):1995-2008.