Pharmacological modulation of macrophage activation phenotypes: the paradigm of dexamethasone.

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Background: Macrophages exist in at least two distinct phenotypes of differentiation/activation: classical/proinflammatory (M1) and alternative/anti-inflammatory (M2) (1). Functional polarization of macrophages has been observed in vivo under physiological and pathological conditions, and may represent an attractive target for pharmacological modulation (2). Given the lack of gold standards for M1 or M2 activation, we polarized human monocyte-derived macrophages using different protocols and performed extensive *in vitro* characterization including the modulation of specific markers by dexamethasone.

Methods: Macrophages were obtained by spontaneous differentiation from human PBMCs isolated by Ficoll density gradient centrifugation, and cultivated in RPMI 1640 medium with 10% FBS. After 7 days, classical (M1) and alternative (M2) phenotypes were generated using specific cytokines (1 μg/ml LPS and 10 ng/ml IFNγ or 20 ng/ml IL-4 and 5 ng/ml IL-13, respectively). Gene expression was analysed using Max SYBR Green qRt-PCR Master Mix. Specific proteins were detected by Western blot.

Results: M1 macrophages preferentially showed long and spindle-shaped morphology, while M2 macrophages were largely round-shaped. We then characterized activation phenotypes by flow cytometry at different time points. When resting macrophages (M0) were incubated with LPS/IFNγ for 4 h, no change was observed in the percentage of cells expressing the M1 surface markers CD68 and CCR2. After longer stimulation (48 h), the percentage of CD68- but not that of CCR2-positive cells doubled compared with M0 (17.8±3.3 vs. 8.9±2.1%, $p<0.05$). This polarization protocol concomitantly down-regulated the expression of M2 markers CD206, CD163 and CX3CR1 compared with M0 (36.4±3.9 vs. 58.0±4.0 for CD206, $p<0.005$; 17.3±4.0 vs 44.1±5.1 for CD163, $p<0.005$; and 16.3±6.4 vs. 35.5±10.0 for CX3CR1). When M2 polarization was induced with IL-4/IL-13 for 48 h, none of the above M2 markers were affected, whereas the percentage of CD206-positive cells was significantly up-regulated after 7 days (78.2±7.2 vs 34.2±12.2, $p<0.05$). As expected, M1 macrophages after 48 h polarization showed higher mRNA levels of TNFα ($p<0.01$), IL-1β ($p<0.005$), COX-2 ($p<0.01$) and VEGF ($p<0.05$) compared with M0, whereas the anti-inflammatory phenotype was associated with increased expression of CD206 ($p<0.05$), COX-1 ($p<0.01$), PPARγ ($p<0.005$) and TGM2 ($p<0.001$; all genes $n=6$). The anti-inflammatory cytokine IL-10 mRNA was unexpectedly more abundant in M1 than M2 macrophages (21.2±8.4 and 1.2±0.4 fold change, respectively; $p<0.05$). Specific qPCR findings (CD206, COX isoforms) were validated by Western Blot analysis. Overnight pretreatment with 10 nM dexamethasone and M2 polarization led to the occurrence of giant cells, particular multinucleated forms of M2 macrophages, and enhanced surface expression of CD163 in both resting and M2-polarized with respect to untreated macrophages (M0: 83.3±5.5 vs. 49.6±9.5, M2: 78.8±8.8 vs. 50.7±11.2; $p<0.05$). Dexamethasone also reversed M1-induced down-regulation of CD163-positive cells (64.4±10.3 vs 23.4±8.3%, $p<0.05$).

Conclusions: M1 and M2 macrophages showed specific profiles of gene expression and surface markers, which were modulated by pharmacological treatment with dexamethasone. Overall, these data suggest that polarized activation protocols have an impact on the functional status of macrophages and are critical to investigate pharmacological macrophage targeting.

References

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