Protocols of human macrophage differentiation and activation: pharmacological modulation by curcumin analogues

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Background: Macrophage activation state can be influenced by a variety of cytokines and microbial products, and may represent an attractive target for pharmacological modulation (1). According to the current framework, macrophages can be polarized into classically (M1) or alternatively (M2) activated cells representing two polar extremes of signals computed by macrophages. Given the lack of gold standards for M1 or M2 activation, we previously demonstrated that the population resulting from spontaneous differentiation has predominance of the M2 over M1 phenotype as measured by flow cytometry, and the expression of M1 vs. M2 markers is not mutually exclusive. Activation with LPS/IFNγ for 48h significantly increases the fraction of surface CD68-expressing cells, the $CD14^+/CD16^-/CD68^+$ subset and cell-bound TNFα levels, whereas expression of CCR2 is unchanged. Expression of the M2 markers CD206, CD163 and CX3CR1 is down-regulated following M1 activation compared with resting. By contrast, alternative activation with IL-4/IL-13 for 48h does not increase M2 markers. Both activation signals induce changes in gene expression profiles as shown by Q-PCR (2). The aims of the present study were: a) test a CSF-1-driven differentiation protocol; b) assess the main cytokines secreted into the medium, and c) determine the modulation of phenotypic markers by curcumin derivatives known to suppress microglial activation through reduced production and release of pro-inflammatory mediators (3).

Methods: Macrophages were differentiated from human PBMCs isolated by density gradient centrifugation, and cultured in RPMI 1640 medium with 10% FBS with or without CSF-1 for 6 days to obtain resting macrophages (M0). Classical (M1) and alternative (M2) phenotypes were generated using specific cytokines (1μg/ml LPS and 10ng/ml IFNγ or 20ng/ml IL-4 and 5ng/ml IL-13, respectively) in the presence or absence of curcumin analogues or dexamethasone. Macrophage phenotypes were determined by flow cytometry using labeled antibodies. Gene expression was analysed using qRt-PCR and phagocytosis was measured using functional assays. The composition of macrophages conditioned media (MCM) was assessed with Luminex technology.

Results: Compared with M0, M2 MCM showed higher levels of anti-inflammatory cytokines including CCL22 and IL-4 ($p<0.05$ and $p<0.001$ vs. M0, respectively), as measured by a Luminex assay. M1 MCM was associated with higher levels of IL-1α ($p<0.05$), IL-1β ($p<0.05$), IL-6 ($p<0.005$), IL-8 ($p<0.001$), MCP-1 ($p<0.005$), VEGF ($p<0.001$) and TNF-α ($p<0.005$; all n=3). After 6 days of CSF-1 and 24h of M1 stimulation, the percentage of CD80- but not that of CCR2-positive cells doubled, whereas expression of the M2 markers CD206, CD163 was down-regulated compared with M0, as observed with spontaneous differentiation. When M2 polarization was induced with IL-4/IL-13 for 24h, however, we observed increased expression of M2 markers compared with M0. Treatment with the curcumin analogue GG9 as well as CLI095, an inhibitor of TLR4 intracellular domain, reversed the M1-induced up-regulation of CD80-expressing cells (20.5±3.5 vs M1 43.45±6.9, $p<0.01$; n=6). A similar effect was maintained with the double positive $CD80^+/CCR2^+$ population. Unlike dexamethasone, which increased the percentage of CD163-positive cells, the curcumin analogue did not affect M2 markers.

Conclusions: M1 and M2 macrophages showed specific profiles of gene expression and surface markers, which were modulated by pharmacological treatment with dexamethasone or a curcumin analogue. 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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