In vitro generation of colonic inflammatory monocyte-like cells and their plasticity into colonic macrophages.

Introduction and objectives:
In mice, under homeostatic conditions, monocytes are continuously recruited into mucosa and differentiate into macrophages (Mφ), a maturation process interrupted in the context of inflammation. We recently identified ex vivo two phenotypically, functionally and molecularly distinct CD14+CD64+ mononuclear phagocyte subsets in inflamed colon of patients with Crohn's disease (CD): the CD163- inflammatory monocyte-like cells (P3) and the CD163+ MF (P4). P3 cells accumulate in proportions correlated with disease severity. The Mφ1 and Mφ2 paradigm proposed that CD14+ monocytes in vitro culture with M-CSF + IFNg differentiate into inflammatory M1 Mφ that can be converted into anti-inflammatory M2 Mφ under the influence of IL4.
The aim of the present study is to identify monocyte culture conditions that would generate surrogates of colonic P3 which could be matured into P4.

Methods: Highly purified CD14+ monocytes (FACS) were first cultured with inflammatory cytokines to generate inflammatory monocytes P3(mo/P3)-like cells and further cultured with anti-inflammatory cytokines to promote their differentiation into Mφ P4 (Mφ/P4)-like cells. Cells were analyzed phenotypically, morphologically, functionally and molecularly.

Results: CD14+CD16- monocytes cultured in the presence of GM-CSF+ IFNg+ IL23 differentiated into a large population (> 85%) of CD14+CD64+CD163- inflammatory mo/P3-like cells that differed from classic monocyte-derived dendritic cells (GM-CSF + IL4). Mo/P3-like cells morphologically resembled colonic P3 cells. Furthermore, like colonic P3, they augmented Th17/Th1 response in effector memory CD4 T cells in an IL1β-dependent manner. Upon exposure to TGFβ and IL10, purified mo/P3-like cells further differentiated into CD163- (Px) and CD163+ Mφ/P4-like cells. The latter displayed Mφ morphology, phagocytosis and induced FoxP3 expression on memory CD4T cells. Gene expression profile segregated mo/P3-like and Mφ/P4-like cells. CD163- Px population appeared more related to Mφ/P4-like cells, and thus might represent an intermediate population in the progression from inflammatory monocytes to Mφ. Finally, Mφ/P4-like cells expressed the gene signature associated with colonic P4 Mφ of CD patients.

Conclusion: The present study identified in vitro culture conditions to generate surrogates of colonic inflammatory monocyte-like cells that accumulate in inflamed CD colon and promote their plasticity into cells that resemble functionally and molecularly colonic Mφ.

Keywords : Crohn's disease, In vitro, Plasticity, macrophages, monocyte-like cells
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