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Original Paper

Two Distinct Cell Populations Are Obtained from Human Blood Monocytes Cultured with M-CSF, GM-CSF and IL-4

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MACROPHAGE-COLONY stimulating factor (M-CSF) (also known as CSF-1) induces monocyte and macrophages migration. It also promotes the differentiation of monocytes into macrophages and thus plays an important role in macrophage homeostasis. In addition, M-CSF stimulates production of cytokines and pinocytosis in fully differentiated macrophages [1, 2]. M-CSF is produced by different normal and activated cell types, as well as by adenocarcinomas of breast, endometrial and ovarian origin. Its cellular effects are mediated through a high affinity membrane receptor, the proto-oncogene c-*fms*. The expression pattern of this receptor is mostly limited to the mononuclear lineage (monocytes, macrophages and also dendritic cells (DC)) but it is also expressed in certain tumour cells. The interaction of M-CSF with c-*fms* leads to activation of multiple signalling events as well as internalisation and degradation of the receptor/ligand complex.

Three main observations suggest an implication of M-CSF in cancer progression. First, M-CSF has been shown to enhance invasiveness of carcinoma cell lines in culture. Second, M-CSF production by human tumours inhibits the functional maturation of DC. Third, high serum levels of M-CSF in breast and ovarian cancer patients, as well as the overproduction of M-CSF by primary breast adenocarcinomas, have been correlated with poor survival.

Most immune cells recruited in tumour tissues possess a macrophage phenotype. These so-called tumour infiltrated macrophages (TIM) have been associated with increased angiogenesis and poor prognosis. Indeed, different cytokines secreted by the intratumoral immune cells can favour neoplastic cell growth and decrease antitumour immune response whereas prolonged immunosuppression is associated with a reduced incidence of breast cancer. These findings suggest that breast cancer might be promoted by immune assistance. Equally, it has been proposed that the pro-angiogenic activity of TIM by inducing the proliferation, the migration and the differentiation of endothelial cells of the vessels, allowed not only a supply of nutrients for tumour cells but also facilitated metastasis. The differentiation pathway of TIM and the precise mechanism by which they allow tumour progression are still poorly understood.

It has been shown that CD34+ progenitors can give rise to mixed populations of DC and macrophages and a common progenitor for both cells has been proposed. Furthermore, immature DC can still be differentiated *in vitro* into macrophages by M-CSF [3]. Therefore, there is no clear frontier between macrophage and the DC lineage. Nevertheless, DC display unique characteristics for antigen presentation, as compared with macrophages: (1) they synthesise high levels of MHC-II molecules; (2) they express specialised receptors thought to potentiate the capture of diverse antigen and their specific delivery to the processing compartments; (3) they efficiently present antigen; and (4) they can prime virgin T lymphocytes *in vitro* and *in vivo*. These characteristics, associated with their distribution throughout the body, allow DC to perform a 'sentinel' function for the immune system.

Whether high levels of M-CSF are able to interfere with the differentiation of DC in the tumour environment and thus impair the immune response to contribute to the tumour progression remains an open question. We tested this potential effect of M-CSF on human cells *in vitro*, using monocyte enriched low density peripheral blood mononuclear cells which can differentiate into DC when cultured in the presence of GM-CSF and IL-4. These DC reached terminal differentiation upon stimulation with TNF-α or LPS [4]. We have shown that monocytes cultured for 7 days with M-CSF, in addition to GM-CSF and IL-4, differentiate into two distinct cell populations that were separated on the basis of their adhesion capability. A first loosely adherent cell population showed DC-like cell characteristics with an accumulation of MHC class II (MHC-II) molecules in lysosomal compartments. Upon stimulation with a high concentration of TNF-α

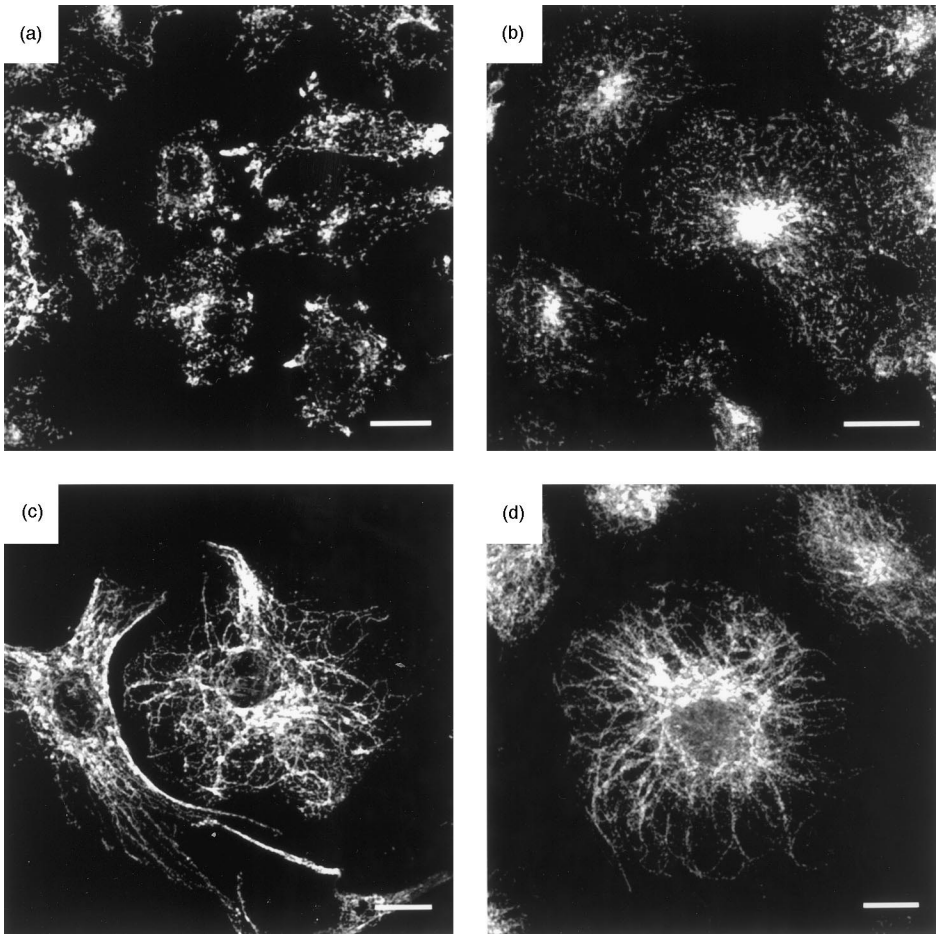

Figure 1. Lysosomal compartments in monocyte-derived cells. Fresh monocytes were cultured for 7 days with GM-CSF and IL4 (a), with the same cytokines and M-CSF (b), (c) or with M-CSF alone (d). All cells were fixed, permeabilised and stained for lamp-1 (lysosomal associated membrane protein-1) cells incubated with GM-CSF, IL4 and M-CSF gave rise to two distinct cell populations, one being loosely adherent (b) and the other strongly adherent (c).

or LPS, the morphology of DC-like cells was partially modified as compared to DC while they elicited a stronger proliferative response of allogeneic T cells in a MLR, via an increased expression of CD86. A second, strongly adherent, cell population had lost most of the normal responsiveness of DC to TNF-α or LPS and displayed a macrophage-like phenotype with tubular lysosomal compartments (Figure 1), few intracellular MHC-II molecules and plasma membrane ruffling. These macrophage-like cells performed better macropinocytosis and elicited less T cell proliferation in MLR than DC. Our results strongly suggest that M-CSF, in the presence of GM-CSF and IL-4, hinders DC differentiation from monocytes and may be immunosuppressive at the tumour site.

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