Peritumoral Administration of Antigen-Unstimulated Bone Marrow-Derived Dendritic Cells Inhibits Tumour Growth

(dendritic cells / tumour vaccines / GM-CSF / IL-4 / HPV 16)

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Abstract. Murine BM cells from B6 mice were grown *in vitro* in medium supplemented with GM-CSF and IL-4 to differentiate DC from DC precursors. After 10 days of culture, approximately 20% of the cell population exhibited the characteristic morphology of BMDC. In cytofluorometric analysis the morphological changes of cells were accompanied by upregulation of the expression of the MHC class II, CD11c, CD80, and CD86 molecules. The BMDC were pulsed with a lysate of syngeneic MK16 carcinoma cells and used for *in vitro* activation of SC. Co-cultivation of the carcinoma lysate-pulsed BMDC with SC induced a proliferative response of the syngeneic SC. Priming of the proliferative responses was more efficient when the BMDC were grown in the presence of GM-CSF and IL-4 for 10 days than for 7 days. The *in vivo* effect of mature, tumour lysate-unstimulated BMDC was examined in mice carrying syngeneic MK16 carcinoma transplants. It has been found that local pretreatment with BMDC inhibits growth of a subsequent challenge inoculum of the MK16 cells. Similarly, treatment of mice carrying small MK16 tumours and of those with MK16 surgical minimal residual disease performed with BMDC significantly inhibited tumour growth. It can be concluded from these results that local concentration of mature BMDC at the tumour site can control the development and growth of the transplanted tumour inoculum.

Dendritic cells (DC) are the most potent antigen-presenting cells (APC) capable of activating naive T cell populations and of inducing primary immune responses. They reside in a resting or immature state in various tissues, where they capture and process antigens. Subsequently, DC migrate to the lymphoid organs, where they are converted into mature DC and efficiently present antigens (Steinman, 1991). DC not only serve as antigen-presenting cells capable of protecting the internal milieu, but also act as regulators of transplant rejection, tissue repair, angiogenesis and initiation as well as maintenance of inflammatory responses.

During the last years it has been possible to generate large quantities of mature DC *in vitro* from their precursors using recombinant cytokines. Granulocyte-macrophage colony-stimulating factor (GM-CSF) has been reported as one of the cytokines able to support both growth and differentiation of DC from DC bone-marrow (BM) precursors (Inaba et al., 1992). Interleukin 4 (IL-4) has also been reported to play a substantial role in the differentiation of DC, probably by suppressing the monocyte/macrophage lineage differentiation from common DC-macrophage precursors (Jansen et al., 1989). In addition, other cytokines (Heufler et al., 1988; Maraskovsky et al., 1996; Saraya and Reid, 1996) and immunoregulatory molecules (Czerniecki et al., 1997; Kalinski et al., 1997) have been proposed to promote DC differentiation and to alter the phenotype and functional properties of DC. It has been found that the capacity of DC to induce tumour immunity correlates with the degree of their differentiation (Labeur et al., 1999).

The aim of this paper was to investigate whether pretreatment with antigen-unstimulated DC can inhibit growth of a subsequent challenge tumour inoculum, whether peritumoral injections of mice carrying small tumours with antigen-unstimulated DC can inhibit these tumours, and whether the percentage of tumour recurrences after surgery can be reduced by local administration of antigen-unstimulated DC.

Material and Methods

Mice

B6 female mice, 2 – 4 months old, were obtained from AnLab Co., Prague, Czech Republic.

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Abbreviations: APC – antigen-presenting cells, B6 – C57BL/6, BM – bone marrow, BMDC – BM-derived DC, DC – dendritic cells, FCS – foetal calf serum, FITC – fluorescein isothiocyanate, GM-CSF – granulocyte-macrophage colony-stimulating factor, IL-4 – interleukin 4, mAb – monoclonal antibody, PBS – phosphate-buffered saline, PE – phycoerythrin, SC – spleen cells.

BM cell preparation

Femurs and tibiae of B6 mice were removed from the surrounding muscle and connective tissue under sterile conditions. The bones were placed in a petri dish containing phosphate-buffered saline (PBS), their epiphyses were cut with scissors and the marrow flushed with RPMI 1640 medium using a 10 cc syringe and a needle. After one wash in RPMI 1640 medium the cells were suspended, counted and diluted to the required concentration with complete RPMI 1640 medium.

Culture of BM cells

We have compared two different methods which are at present being used for *in vitro* generation of DC from their BM precursors, the method described by Fields et al. (1998) and that described by Lutz et al. (1999). Both of the methods were slightly modified.

Briefly, in the method 1 (Fields et al., 1998), the BM-derived cells were seeded in a concentration of 1.0×10^6 per ml and grown in complete RPMI 1640 medium, i. e. in the RPMI 1640 medium supplemented with gentamycin (Sigma-Aldrich, St. Louis, MO), 2 nM L-glutamine (Sevac, Prague, Czech Republic), 10^{-5} M mercaptoethanol (Calbiochem, La Jolla, CA), and 10% heat-inactivated foetal calf serum (FCS) (BioClot, Ltd., Aidenbach, Switzerland). The complete RPMI 1640 medium was further supplemented with 10 ng/ml granulocyte-macrophage colony-stimulating factor (GM-CSF) (Sigma-Aldrich, St. Louis, MO) and 10 ng/ml interleukin 4 (IL-4). The IL-4 was produced in our

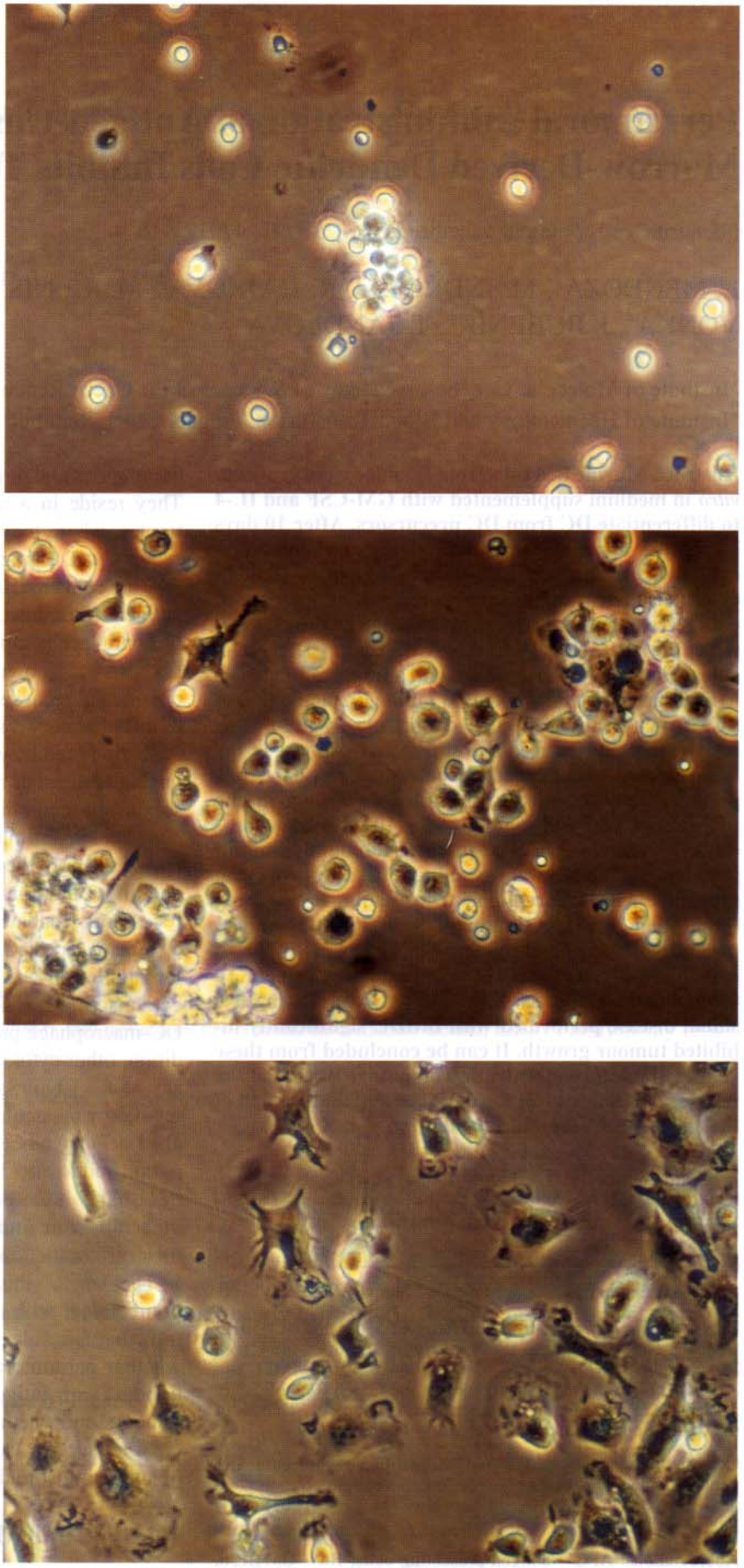

Fig. 1. Differentiation of DC from their precursors by 10 days of culture in the medium supplemented with GM-CSF and IL-4. (A) Fresh BM cells; (B) BMDC grown for 5 days; (C) BMDC grown for 10 days (phase contrast micrographs ×100).