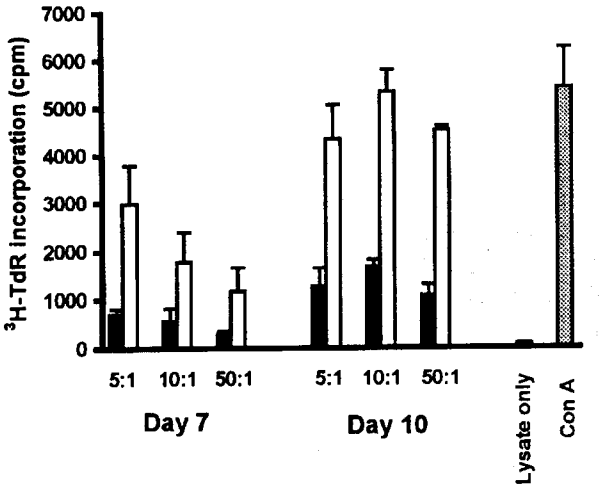

Fig. 3. Higher SC proliferative responses (mean ± SD) after priming with 10-day cultured BMDC pulsed with irradiated MKl6 lysate than after priming with 7-day cultured BMDC pulsed with irradiated MKl6 lysate. SC/BMDC ratio 5 : 1, 10 : 1, 50 : 1. Black bars – BMDC; white bars – BMDC pulsed with the irradiated MKl6 lysate. As control samples, SC co-cultured for 4 days with irradiated MK16 lysate (lysate only), and SC co-cultured with 2 g/ml Concanavalin A (Con A) for 72 h were used.

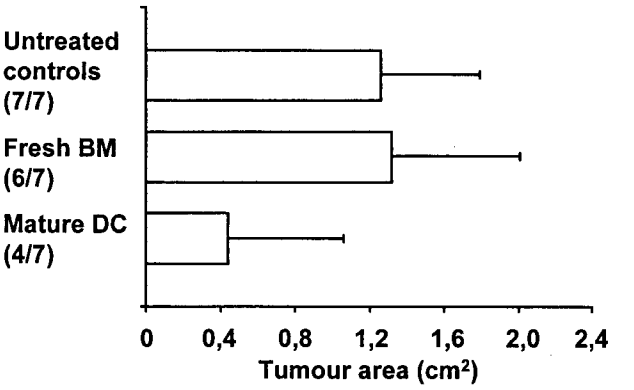

Fig. 5. Peritumoral (s.c.) treatment of 5-day MKl6 tumours with 3.0×10^6 BMDC grown for 10 days in the medium supplemented with GM-CSF and IL-4. On day 52, the size of tumours was compared and found to be significantly different between the DC group and untreated controls ($P < 0.05$). No significant difference was found between the untreated controls and mice injected s.c. with 3.0×10^6 fresh BM cells ($P > 0.05$). The number of tumour-bearing mice/ total number of mice is given in parentheses.

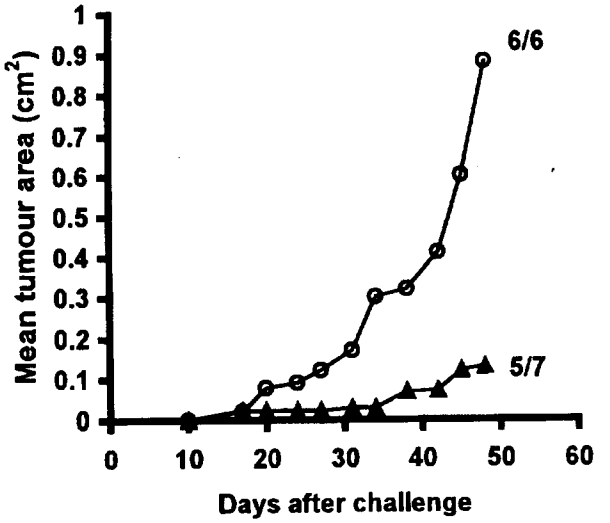

Fig. 4. The effect of local BMDC pretreatment on s.c. growth of MKl6 carcinoma transplants in syngeneic mice. Black triangles – BMDC; open circles – untreated controls. The number of tumour-bearing mice/total number of mice is given. Significant differences were observed between growth curves of the MKl6 tumour in BMDC-pretreated and untreated control mice ($P < 0.05$).

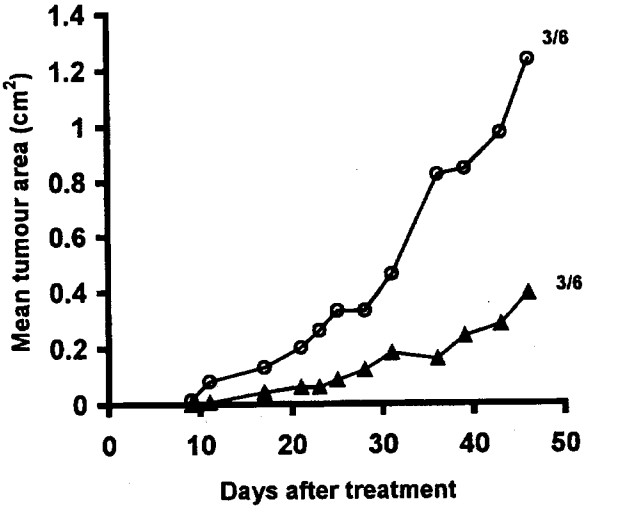

Fig. 6. Local (s.c.) treatment of surgically induced minimal residual MKl6 tumour disease with 3.0×10^6 BMDC grown for 10 days in the medium supplemented with GM-CSF and IL-4. Black triangles – operated + BMDC-injected group; open circles – operated only group. A significant difference was found between growth curves of the MKl6 tumour in the operated plus BMDC-injected and operated only group ($P < 0.05$).

recorded. For statistical analysis the SigmaStat Package program was used.

Results

BMDC pulsed with MKl6 carcinoma lysate were examined for their ability to stimulate proliferative responses of syngeneic SC. Substantially higher proliferative responses were obtained after priming with 10-day BMDC as compared to those induced by 7-day BMDC (Fig. 3). Therefore, for *in vivo* experiments, the 10-day cultured BMDC were used.

In an attempt to evaluate the role of local BMDC in the defence against tumours, B6 mice were pretreated twice on days 0 and 14 with 3.0×10^6 antigen-unstimulated BMDC grown for 10 days in the medium supplemented with GM-CSF and IL-4. The mice were challenged on day 21 with a dose of 1.0×10^5 syngeneic MKl6 carcinoma cells. As shown in Figure 4, on day 48 after MKl6 challenge the mean area of tumours (± SE) in the BMDC-pretreated mice was 0.13 (± 0.09) cm^2 whereas in the untreated controls it was 0.88 (± 0.39) cm^2 ($P < 0.05$). The mean survival of the experimental mice was 133.0 ± 42.5 days and of the untreated controls 68.0 (± 21.9) days ($P < 0.05$).

Local treatment of 5-day MKl6 tumours with 3.0×10^6 antigen-unstimulated BMDC, grown for 10 days in the medium supplemented with GM-CSF and IL-4, revealed a difference ($P < 0.05$) between the mean area of the tumours in the BMDC-treated group 0.44 (± 0.23) cm^2 as compared to the untreated control group 1.26 (± 0.20) cm^2 on day 52 after challenge (Fig. 5).

Mature antigen-unstimulated BMDC were also used for treatment of surgical residual tumour disease. B6 mice were inoculated with 1.0×10^6 MKl6 carcinoma cells and one month after inoculation, when the tumours have reached 8–12 mm in diameter, the tumours were completely excised. Three days after the operation, the mice were randomly divided into two groups. The experimental group was injected in the vicinity of the excised tumour with 3.0×10^6 BMDC grown for 10 days in the medium supplemented with GM-CSF plus IL-4 (operated + vaccinated) and the control group was left as untreated (operated only). On day 46, the mean area of the recurrent MKl6 tumours was 0.40 (± 0.31) cm^2 in the operated + vaccinated group and 1.23 (± 0.60) cm^2 in the operated only group ($P < 0.05$) (Fig. 6).

Taken together, these results suggest that injections of antigen-unstimulated BMDC at the site of the growing tumour can influence tumour growth.

Discussion

Two different methods for generation of murine mature DC from their precursors were previously described by Fields et al. (1998) and by Lutz et al. (1999). We have compared the two methods after slight modification and have chosen the modified method of Fields et al. (1998) for further experiments. After 10-day cultivation, approximately 20% of cells with the morphology of DC were observed in the BM cell cultures and a substantial upregulation in the expression of MHC class II, CD80, CD86 and CD11c was observed. We have found that the antigen-presenting capacity of BMDC grown in the medium supplemented with GM-CSF and IL-4 for 10 days is substantially higher than that of BMDC grown in the same medium for 7 days.

The *in vivo* effects of MKl6 lysate-unpulsed mature BMDC were investigated in experiments utilizing prophylactic administration of BMDC, in therapeutic experiments utilizing small (5-day) tumours, and in surgically induced minimal residual tumour disease. It has been found that both prophylactic and therapeutic experiments that use local injections of mature, exogenous BMDC at the tumour site or its vicinity can substantially reduce the number of takes of the tumour transplants as well as inhibit their growth. Our results are in agreement with clinical studies which reported a correlation between the concentration of DC at the site of a growing tumour or in its vicinity and the prognosis of patients with oesophageal carcinoma (Matsuda et al., 1990), colorectal adenocarcinoma (Ambe et al., 1989), gastric carcinoma (Tsujitani et al., 1990), nasopharyngeal carcinoma (Nomori et al., 1986), lung carcinoma (Furukawa et al., 1985), thyroid (Schroder et al., 1988) and prostate cancer (Bigotti et al., 1991). However, our paper brings the first evidence that administration of exogenous, antigen-unstimulated, mature BMDC at the site of the growing tumour can inhibit tumour growth.

Acknowledgement

We thank Dr. E. Šloncová, Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, for her help with microphotographs.

References

Ambe, K., Mori, M., Enjoji, M. (1989) S-100 protein-positive dendritic cells in colorectal adenocarcinomas. Distribution and relation to the clinical prognosis. *Cancer* **63,** 496-503.

Bigotti, G., Coli, A., Castagnola, D. (1991) Distribution of Langerhans cells and HLA class II molecules in prostatic carcinomas of different histopathological grade. *Prostate* **19,** 73-87.

Bubeník, J. (1999) Dendritic cell-based cancer vaccines. *Folia Biol. (Praha)* **45,** 71-74.

Bubeník, J., Šímová, J., Hájková, R., Sobota, V., Jandlová, T., Šmahel, M., Sobotková, E., Vonka, V. (1999) Interleukin 2 gene therapy of residual disease in mice carrying tumours induced by HPV 16. *Int. J. Oncol.* **14,** 593-597.

Czerniecki, B. J., Carter, C., Rivoltini, L., Koski, G. K., Kim, H. I., Weng, D. E., Roros, J. G., Hijazi, Y. M., Xu, S., Rosenberg, S. A., Cohen, P. A. (1997) Calcium ionophore-treated peripheral blood monocytes and dendritic cells rapidly display characteristics of activated dendritic cells. *J. Immunol.* **159,** 3823-3837.

Fields, R. C., Osterholzer, J. J., Fuller, J. A., Thomas, E. K., Geraghty, P. J., Mulé, J. J. (1998) Comparative analysis of murine dendritic cells derived from spleen and bone marrow. *J. Immunother.* **21,** 323-339.

Furukawa, T., Watanabe, S., Kodama, T., Sato, Y., Shimosato, Y., Suemasu, K. (1985) T-zone histiocytes in adenocarcinoma of the lung in relation to postoperative prognosis. *Cancer* **56,** 2651-2656.

Heufler, C., Koch, F., Schuler, G. (1988) Granulocyte/macrophage colony-stimulating factor and interleukin-1 mediate the maturation of epidermal Langerhans cells into potent immunostimulatory dendritic cells. *J. Exp. Med.* **167,** 700-705.

Inaba, K., Inaba, M., Romani, N., Aya, H., Deguchi, M., Ikehara, S., Muramatsu, S., Steinmann, R. M. (1992) Generation of large numbers of dendritic cells from mouse bone marrow cultures supplemented with granulocyte/macrophage colony-stimulating factor. *J. Exp. Med.* **176,** 1693-1702.

Jansen, J. H., Wientjens, G. J., Fibbe, W. E., Willemze, R., Kluin-Nelemans, C. (1989) Inhibition of human macrophage colony formation by interleukin 4. *J. Exp. Med.* **170,** 577-582.

Julius, M. H., Simpson, E., Herzenberg, L. A. (1973) A rapid method for the isolation of functional thymus-derived murine lymphocytes. *Eur. J. Immunol.* **3,** 645-649.

Kalinski, P., Hilkens, C. M., Snijders, A., Snijdewint, F. G., Kapsenberg, M. L. (1997) IL-12 deficient dendritic cells, generated in the presence of prostanglandin E_2, promote type 2 cytokine production in maturing human naive T helper cells. *J. Immunol.* **159,** 28-35.

Karasuyama, H., Melchers, F. (1988) Establishment of mouse cell lines which constitutively secrete large quantities of interleukin-2, 3, 4 or 5 using modified cDNA expression vectors. *Eur. J. Immunol.* **18,** 97-104.

Labeur M. S., Roters, B., Pers, B., Mehling, A., Luger, T. A., Schwarz, T., Grabbe, S. (1999) Generation of tumor immunity by bone marrow-derived dendritic cells correlates with dendritic cell maturation stage. *J. Immunol.* **162,** 168-175.

Lutz, M., Kukutsch, N., Ogilvie, A., Rossner, S., Koch, F., Romani, N., Schuler, G. (1999) An advanced culture method for generating large quantities of highly pure dendritic cells from mouse bone marrow. *J. Immunol. Meth.* **223,** 77-92.

Maraskovsky, E., Brasel, K., Teepe, M., Roux, E. R., Lyman, S. D., Shortman, K., McKenna, H. J. (1996) Dramatic increase in the numbers of functionally mature dendritic cells in FLT3 ligand-treated mice: multiple dendritic cell subpopulations identified. *J. Exp. Med.* **184,** 1953-1962.

Matsuda, H., Mori, M., Tsujitani, S., Ohno, S., Kuwano, H., Sugimachi, K. (1990) Immunohistochemical evaluation of squamous cell carcinoma antigen and S-100 protein-positive cells in human malignant esophageal tissues. *Cancer* **65,** 2261-2265.

Nomori, H., Watanabe, S., Nakajima, T., Shimosato, Y., Kameya, T. (1986) Histiocytes in nasopharyngeal carcinoma in relation to prognosis. *Cancer* **57,** 100-105.

Saraya, K., Reid, C. D. (1996) Stem cell factor and the regulation of dendritic cell production from $CD34^+$ progenitors in bone marrow and cord blood. *Br. J. Haematol.* **93,** 258-264.

Schroder, S., Schwarz, W., Rehpenning, W., Loning, T., Bocker, W. (1988) Dendritic/Langerhans cells and prognosis in patients with papillary thyroid carcinomas. Immunocytochemical study of 106 thyroid neoplasms correlated to follow-up data. *Am. J. Clin. Pathol.* **89,** 295-300.

Steinman, R. M. (1991) The dendritic cell system and its role in immunogenicity. *Ann. Rev. Immunol.* **9,** 271-296.

Tsujitani, S., Kakeji, Y., Watanabe, A., Kohnoe, S., Maehara, Y., Sugimachi, K. (1990) Infiltration of dendritic cells in relation to tumor invasion and lymph node metastasis in human gastric cancer. *Cancer* **66,** 2012-2016.

Vlk, V., Rössner, P., Indrová, M., Bubeník, J., Sobota, V. (1998) Interleukin 2 gene therapy of surgical minimal residual tumour disease. *Int. J. Cancer* **76,** 115-119.