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Short communication

Liposomes as cytokine-supplement in tumor cell-based vaccines

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Abstract

Subcutaneous vaccination of C57bl/6 mice with irradiated B16 melanoma cells supplemented with liposomal interleukin-2 (IL2) or murine interferon-gamma (mIFNγ), resulted in systemic protection in 50% of the animals, against a subsequent tumor cell challenge in a dose dependent manner. The protective efficacy was comparable to the efficacy of cytokine gene-modified cells as tumor vaccine, whereas irradiated B16 cells supplemented with soluble cytokine did not result in protective responses. In vivo evidence was obtained that the beneficial effects mediated by liposome incorporation of the cytokine are the result of a depot function of the liposomal cytokine supplement at the vaccination site. In can be concluded that liposomal delivery of cytokines offers an attractive alternative to cytokine-gene transfection of tumor cells for therapeutic vaccination protocols.

Keywords: Liposomes; Vaccination; Interferon-γ; Interleukin-2; Melanoma

In the application of autologous cells as tumor vaccines, administration of cytokines as supplement may be important in expanding the vaccine-induced response against tumor cells (Mitchell, 1992, 1995; Restifo and Sznol, 1997). Interest currently lies in the utility of interferon-gamma (IFNγ) and interleukin-2 (IL2) for this purpose. IFNγ is an attractive cytokine to use as adjuvant considering its ability to induce expression of the major histocompatibility complex and activation of macrophages and natural killer cells. IL2 is a

Abbreviations: HPLC, high performance liquid chromatography; IL2, interleukin-2; mIFNγ, murine interferon-gamma; s.c., subcutaneous.

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cytokine known for its ability to stimulate the proliferation of T cells, regulating the immune response. The clinical use of these proteins, however, is limited by a rapid clearance and a fast biodegradation, non-specific tissue distribution and systemic toxicity when administered intravenously at therapeutic doses (Quesada et al., 1986; Redman et al., 1990; Atkins and Trehu, 1995). Since most cytokines are paracrine factors working physiologically in direct vicinity of their originating cell, the development of systems slowly releasing the cytokine in question at the site of antigen presentation is of considerable interest. One possible approach is the use of cytokine-gene transfected autologous tumor cells (Gansbacher et al., 1990; Maass et al., 1995; Zatloukal et al., 1995; Abdel Wahab et al., 1997). The application of this approach is limited by several factors, including labor intensity and variability between tumor cells of different patients in terms of transfection efficiency. Encapsulation of proteins in liposomes is technically less complex and may also give a sustained presence of the entrapped cytokine at the vaccination site, due to a depot effect.

To demonstrate that liposomes remain at the local injection site and slowly release the cytokine in vivo, a biodistribution study was performed with ^{125}I-labeled murine IFNγ (mIFNγ) encapsulated in liposomes and mIFNγ encapsulated in [^{3}H]cholesteryloleylether-labeled liposomes composed of egg-phosphatidylcholine and egg-phospatidylglycerol (in a molar ratio 9:1, the total lipid concentration was 40 mM). Unsized liposomes were injected subcutaneously (s.c.) at the flank of C57bl/6 mice and the animals were killed at predetermined time points. Relevant organs, injection site and blood were assayed for radioactivity. As reflected by the ^{3}H-data, the unsized liposomes remained at the injection site for at least 7 days. However, the ^{125}I-data indicate that a large fraction of mIFNγ was released from the liposomes and eliminated from the injection site following a 'burst' release period of about 4 h. The release profile was more sustained from 4 hours on up to 7 days. In contrast, soluble [^{125}I]mIFNγ is rapidly cleared within the first hours after administration. Comparison of the area under the curves of the percentage of injected dose over time of liposomal and free (i.e. soluble) radiolabeled mIFNγ revealed that liposomal encapsulation of mIFNγ results in a 4-fold increase in residence time of mIFNγ. Interestingly, the cytokine amount released by the mIFNγ-liposomes in vivo during the first 24 h, appeared roughly equivalent to the amount produced by mIFNγ-gene transfected B16 tumor cells as reported in an earlier study (Kircheis et al., 1998). At any time point evaluated, only a low localization of radio-label was found in blood, liver, spleen, kidneys, and inguinal lymph nodes.

The adjuvanticity of liposomal cytokine formulations was investigated in an established tumor vaccination model. C57bl/6 mice were vaccinated twice on the right flank at 14 and 7 days before challenge with 1×10^5 syngeneic B16 melanoma cells. The liposome-cytokine depot concept was investigated for two cytokines: mIFN and IL2. The cytokine supplemented cell vaccine consisted of irradiated cytokine-gene transfected B16 cells or 2×10^5 irradiated B16 cells admixed with soluble or liposomal cytokine. The plasmid DNA encoding for the cytokine-genes was introduced in the melanoma cells by receptor-mediated adenovirus enhanced gene delivery (AVET) (Wagner et al., 1992; Zatloukal et al., 1993; Kircheis et al., 1998). Vaccination with IFNγ-gene transfected tumor cells yielded complete protection against a lethal tumor cell challenge in seven out of 16 animals. Irradiated B16 cells supplemented with the liposomal mIFNγ formulation induced similar protection at comparable doses. A clear dose optimum was observed for the liposomal formulation. The bell-shaped dose response curve was also observed for mIFNγ-gene transfected cells. Liposomal supplements containing too low doses of cytokines or gene-transfected tumor cells producing too low amounts of cytokines, did not induce adequate systemic immune responses, whereas too high doses were not successful either, probably due to the failure to generate tumor specific CTLs as was hypothesized by Schmidt et al. (1995). Supplementing the irradiated tumor cell vaccine with soluble mIFNγ, did not result in a significant protection against tumor growth (data submitted for publication).

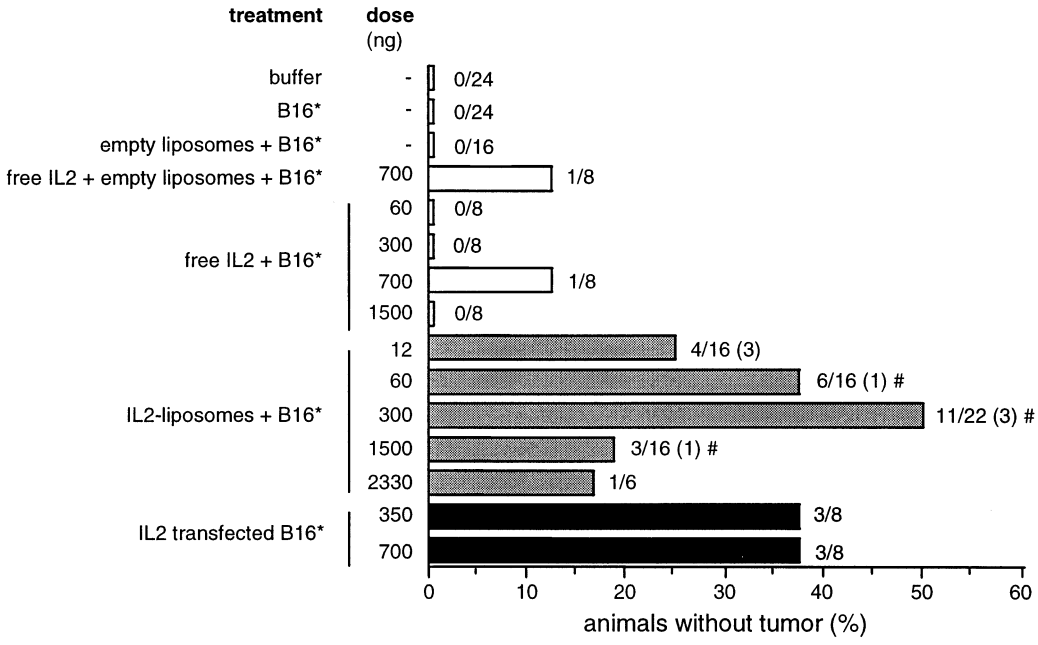

Fig. 1. Protection of mice against a lethal challenge with B16 melanoma cells. Columns, percentage of animals without tumor at day 80 after the tumor challenge. Ratios, numbers of surviving animals/total number of challenged animals. Numbers in parentheses, occurrence of vitiligo. To statistically compare vaccination using liposomal IL2 + irradiated B16 cells with vaccination using soluble IL2 + irradiated B16 cells, these groups were compared by the Mantel–Haenszel test. # $P < 0.001$ (from Koppenhagen et al., 1998).

Remarkably, most surviving animals immunized with the cell-based vaccine supplemented with liposomal mIFNγ, as well the animals vaccinated with the mIFNγ-gene transfected cells showed pronounced vitiligo at both the vaccination and the contralateral challenge sites. This phenomenon was absent in any of the non-protected mice. It is hypothesized that the occurrence of vitiligo is due to an immune response directed against shared antigen(s) on melanocytes and melanoma tumor cells, resulting in killing of either cell type (Berd et al., 1996; Rosenberg and White, 1996). The results obtained in case of IL2 liposomes supplement and IL2-gene transfected cells are shown in Fig. 1(Koppenhagen et al., 1998). Results are comparable to those obtained with mIFNγ as the cytokine.

In conclusion, the results indicate that cytokine-liposomes can function as a cytokine depot at the site of antigen presentation. Irradiated B16 cell admixed with liposomal IL2 or liposomal mIFNγ were effective in evolving a protective immune response. As the release characteristics of the cytokine-liposomes can be tailored and liposomes are feasible for industrial-scale production, they offer an off-the-shelf product as an attractive alternative to cytokine-gene transfection of tumor cells for anti-tumor vaccination therapy.

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