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IMMUNOTHERAPY

Dendritic-tumor cell hybrids induce tumor-specific immune responses more effectively than the simple mixture of dendritic and tumor cells

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Abstract

Background aims. Dendritic cell (DC)-tumor cell hybrids have been used clinically in cancer immunotherapy, but their advantage over the simple mixture of tumor cells and DCs is still a matter of controversy. In this study, we compared DC-tumor cell hybrids with the non-fused mixture of DC and tumor cells directly in their ability to induce a specific immune response. *Methods.* Hybrids were obtained by electrofusion of tumor cells and monocyte-derived DCs. Cell phenotype was evaluated by flow cytometry and antigen-presenting ability by co-culture with syngeneic T cells followed by tetramer analysis and interferon (IFN)-γ ELISPOT. *Results.* Less than half the cells in the mixture expressed DC co-stimulatory molecules. Furthermore, DCs in the mixture had significantly lower expression of MHC class I molecules than DCs in the fusion. Conversely, nearly all CD11c$^+$Her2/neu$^+$ hybrids expressed CD80, CD86, CD83, HLA-DR and MHC class I from both tumor cells and DCs. Using tumor cells constitutively expressing a cytomegalovirus (CMV) antigen, we show that expansion of CMV-specific cytotoxic T lymphocytes (CTLs) restricted by DCs' MHC class I molecules was higher when DC-tumor hybrids were the stimulators. Furthermore, only hybrids stimulated CTLs to produce IFN-γ in response to CMV-positive target cells. *Conclusions.* These data show the superiority of DC-tumor cell hybrids over their simple mixture as T-cell stimulators. Hybrids expressed more co-stimulatory and MHC molecules, induced higher antigen-specific T-cell expansion and were the only cells able to induce IFN-γ–producing antigen-specific T cells. Thus, these data offer further support for cancer immunotherapeutic approaches using DC-tumor cell hybrids.

Key Words: *cancer vaccines, cell fusion, dendritic cells, hybrid cells, immunotherapy*

Introduction

Dendritic cells (DCs) are professional antigen-presenting cells frequently used in immunotherapy protocols because they are key cells to activate the adaptive immune response [1]. To induce antitumor responses, DCs must be loaded with tumor antigens, a process that can be achieved by loading the cells with peptides or by transfecting or transducing DCs with the antigen cDNA or mRNA [2–4]. However, finding immunologically relevant antigens is challenging and not always necessary because there are protocols for loading DCs with a pool of antigens [5,6]. This can be achieved by loading dendritic cells with tumor cell bulk products, as in the case of tumor lysates, tumor cell total mRNA, necrotic debris, exosomes, apoptotic

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bodies and live tumor cells [7–10]. Another strategy consists of fusing dendritic cells with tumor cells, generating hybrids that allow virtually all tumor antigens to be presented with the appropriate co-stimulation [11–13]. The fusion can be achieved even by using polyethylene glycol [14] or by applying an electric pulse to the cells (electrofusion) [15].

It was already shown that DC-tumor cell hybrids can express MHC class I, class II and co-stimulatory molecules, induce antitumor responses and cause the decrease of tumor size [12,16–18]. Also, clinical trials indicated that vaccination with DC-tumor cell hybrids may change the natural history of the disease in patients with renal cell carcinoma, metastatic melanoma, breast cancer and other malignant tumors [19–21], showing the clinical potential of these hybrids in antitumor therapeutic approaches.

Comparisons of different ways to load DC with tumor antigens showed that tumor cell fusion was better than whole lysates, apoptotic or necrotic cells [22–25]. However, it is still controversial whether the DC-tumor cell hybrids are indeed better inducers of an immune response than the simple mixture of DCs and tumor cells. Draub et al., for example, directly compared the antigen-specific T-cell activation induced by the mixture of DCs and tumor cells, by polyethylene glycol–induced or by electrofused hybrids and showed very similar efficiency among the three groups [26]. The process of hybrids generation adds more complexity to the protocols, and thus, it is important to address whether the fusion really enhances the ability of the cells to induce an antitumor immune response.

This work aimed to compare the antigen presentation ability of the hybrids to that of mixtures of DCs and live tumor cells. For this purpose, DCs were obtained from the differentiation of peripheral blood monocytes of healthy donors, and different tumor cells were used as fusion partners [27,28]. To evaluate the generation and phenotype of the hybrid cells, we used the cell line MDA-MB-231, which is a breast carcinoma cell line that expresses Her-2/neu, an epidermal growth factor receptor chain that is expressed by carcinoma cells but not by dendritic cells [29]. To determine the ability of the hybrids or the mixture to induce an antigen-specific response, we used artificial target cells expressing HLA-A2 and genetically programmed to express the pp65 human cytomegalovirus (CMV) protein. This antigen may be presented in the context of either HLA-A2 or HLA-B07 [30,31], and DCs were generated from HLA-A2$^+$HLA-B7$^+$ donors. Using this model system, we show that DC-tumor cell hybrids are effective antigen-presenting cells that have the unique ability to activate interferon (IFN)-γ–producing T cells specific to the antigen presented in target cells.

Methods

Peripheral blood mononuclear cell isolation and DC generation

Leukapheresis chambers were collected after participants gave written informed consent and after approval of the ethics committee of the Institute of Biomedical Sciences and the ethics committee of the Hannover Medical School. Peripheral blood mononuclear cells (PBMCs) were separated by gradient centrifugation over Ficoll-Paque (GE Healthcare). PBMCs were seeded in 75-cm^2 cell culture flasks and incubated overnight at 37°C and 5% CO_2. After overnight incubation, nonadherent cells were removed, and the adherent cells were cultured in AIM-V supplemented with granulocyte macrophage colony-stimulating factor (50 ng/mL; Peprotech) and interleukin (IL)-4 (50 ng/ml; Peprotech). After 5 days, the cells received a maturation stimulus with tumor necrosis factor-α (50 ng/ml; Peprotech). Mature DCs (mDCs) were obtained 48 hr after activation.

To stimulate T cells in the microculture, DCs were generated from isolated CD14$^+$ cells cultured for 7 days in X-VIVO 15 medium (Lonza), supplemented with recombinant granulocyte-macrophage colony-stimulating factor (50 ng/mL; Cellgenix) and IFN-α (1000 U/mL; PBL IFNSource). Cytokines were replenished every 3 days.

Generation of DC-tumor cell hybrids

After differentiation, DCs were harvested, washed and resuspended in a sterile 5% glucose solution to a concentration of 4×10^6 cells/mL. Tumor cells were also resuspended in a sterile 5% glucose solution to a concentration of 4×10^6 cells/mL. MDA-MB-231 cells were fused with DCs by mixing 1×10^6 cells of each type and applying an electric pulse of 1000 V/cm at 25 μF, after being aligned in an inhomogeneous electrical field (62.5 V/cm) for 15 s. KA2 cells were fused with DCs by applying an electric pulse of 1000 V/cm at 25 μF, with a constant resistance of 600 Ω, using the Gene Pulser Xcell Electroporation Systems (BioRad). After resting for 5 min in the electroporation cuvette, 500 μL of a relaxation buffer (100 mmol/L KCL, 3 mmol/L NaCl, 1.25 mmol/L ethylenediaminetetraacetic acid, 10 mmol/L piperazine-N,N′-bis(2-ethanesulfonic acid) (PIPES), 0.5 mmol/L adenosine triphosphate, adjusted to pH 6.8) were added. After being kept in the cuvette for 5 min longer, cells were transferred to a 15-mL tube, centrifuged and resuspended in culture medium. After at least 30 min in the culture medium to recover from the electroporation process, cells were harvested and stained with antibodies for different surface markers. For the analysis of cell mixtures without fusion, the cells were mixed in the same conditions