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Tissue localization of tumor antigen-loaded mouse dendritic cells applied as an anti-tumor vaccine and their influence on immune response

Joanna Rossowska, Elżbieta Pajtasz-Piasecka, Anna Szyda, Natalia Ziętara, Danuta Duś

Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland

Abstract: The recognition, internalization and intracellular processing of antigen are the main functions of dendritic cells (DCs). In the course of these processes, DCs differentiate and acquire the ability to produce cytokines responsible for polarization of the immunological response. Therefore, vaccination with tumor antigen-loaded DCs is one of the most promising approaches to induce tumor-specific immune response. The purpose of this study was to analyze the migratory abilities, from an injection site to tumor-draining lymph nodes (tLN), of DCs applied as an anti-tumor vaccine and their capacity for immune response activation. Mouse DCs of the established JAWS II cell line transduced with EGFP gene or ex vivo bone marrow-isolated DCs (BM-DCs) stained with intravital CFDA dye were loaded with MC38 colon carcinoma tumor lysate (TAg) and then administered peritumorally to MC38 tumor-bearing C57BL/6 mice. On the first, third, fifth and seventh days after injection the tumors, tLNs and spleens were examined. The TAg-loaded DCs migrated more effectively to the tLNs than did the unloaded control DCs; however, the majority of them remained in the tumor vicinity. Immunohistological analysis of the tumor tissues demonstrated that only TAg-loaded DCs activated an immune response. Seven days after DCs vaccine administration, numerous necrotic areas and some apoptotic bodies were observed in the tumor tissue. However, the anti-MC38 tumor cytotoxic activity of spleen and tLN cells from mice treated with both TAg-loaded and unloaded DCs reached a maximum on the fifth day after DC injection. Concluding, TAg-loaded DCs migrated more efficiently to tLNs and were more effective activators of local (but not systemic) cellular immune response than were unloaded DCs. We hypothesize that only the application of TAg-loaded DCs to tumor-bearing mice as an adjuvant supporting chemotherapy may activate a more effective anti-tumor response.

Key words: Mouse colon carcinoma MC38 - Tumor antigen-loaded dendritic cells - DC migration - T cell cytotoxicity - IFN-γ production

Introduction

Understanding the role the immune system in the host defense against cancer is fundamental for both present and future attempts of anti-cancer immunotherapy. Dendritic cells (DCs) are among the most effective immune response activators. The main function of immature DCs is antigen recognition, internalization, and its intracellular processing. Immature DCs circulating in the blood survey peripheral tissues and are recruited to inflammatory sites for antigen uptake. Antigen-loaded DCs gain the ability to migrate through lymphatics into draining lymphoid tissues [1,2]. During migration, DCs may activate naive T lymphocytes [3] which, after differentiation, acquire the ability to migrate from the lymph node to an inflammatory site.

It has been demonstrated that exogenous DCs loaded with a tumor-specific antigen effectively prime T cells [4,5]. In animal tumor models, DCs loaded with tumor antigen induced anti-tumor response and even a regression of established tumor [6]. However, clinical trials of anti-cancer immunotherapy with antigen-loaded DCs varied and

Correspondence: E. Pajtasz-Piasecka, Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Laboratory of Experimental Anticancer Therapy, R. Weigla Str. 12, 53-114 Wroclaw, Poland; tel.: (+4871) 3371172 ext. 373, fax.: (+4871) 3371172 ext. 366, e-mail: pajtasz@iitd.pan.wroc.pl

were less successful than expected [7]. It seems that much is still to be learned about DCs *in vivo* behavior and the factors that control their ability to induce an effective immune response.

In this study a detailed analysis of the migratory capacities of peritumorally injected DCs loaded with MC38 colon carcinoma tumor lysates (TAg-loaded DCs) was performed. To determine whether TAg-loaded DCs are efficient activators of immune response, the effector activities of spleen and tumor-draining lymph node (tLN) cells as well as MC38 tumor tissue infiltration with immune cells were estimated.

Materials and methods

Mice. The experiments were performed using 6- to 8-week-old female C57BL/6 mice which were finally sacrificed according to properly established protocols and government regulations (permission of the Local Ethics Committee, 2004).

Cell culture. RPMI 1640 culture medium (Gibco) was supplemented with 100 U/mL penicillin, 100 U/mL streptomycin, 2 mmol/L L-glutamine, 1 mmol/L sodium pyruvate, and 50 μmol/L 2-mercaptoethanol (complete medium, CM). Cell of an established mouse colon carcinoma cell line (MC38/0) [8] were cultured in CM supplemented with 5% FCS. C57BL/6 mouse bone marrow-derived dendritic cells of the JAWS II line (ATCC Cat. No. CRL-11904) were maintained in a 1:1 mixture of alpha MEM (Gibco) and CM supplemented with 10% FCS and 5 ng/mL GM-CSF (Strathman). JAWS II cells transduced with a retroviral vector carrying enhanced green fluorescent protein gene (EGFP) and *neo*^{r} gene as a selection marker (JAWS II/EGFP) were maintained under the same culture conditions as the JAWS II cells.

Generation of bone marrow-derived DCs (BM-DCs). Briefly, the long bones of the female C57BL6 mice were dislodged and the remaining tissue and muscles were removed with sterile gauze. The ends of the bones were cut with sterile scissors and the bone marrow was flushed from the femurs and tibias with 5% RPMI. The bone marrow was washed three times and the cells were re-suspended in CM supplemented with 10% (v/v) FCS. The cells were cultured in 75-cm^2 flasks at a concentration of 8×106 cells per flask in 10 ml of 10% CM supplemented with 40 ng/mL recombinant murine GM-CSF and IL-4. The source of mouse IL-4 was X63mIL4 plasmacytoma cell supernatant used in a concentration of 5-10%, corresponding to 100 U/ml. The CM was replaced every second day. After six days the BM-DCs were used for further tests.

DCs stimulation with tumor antigen. MC38/0 lysate (called here tumor antigen: TAg) was prepared by five cycles of freezing in liquid nitrogen and thawing at 37°C. Then the mixture was sonicated for two hours. The lysate was frozen and stored at -20°C. Immature DCs (BM-DCs and/or JAWS II/EGFP cells) were seeded in 75 cm^2 flasks and then loaded with TAg for 24 hours (0.1 ml of TAg was added to 1 ml cell suspension). After antigen loading, the BM-DCs were stained with intracellular dye CFDA (Molecular Probes) according to the producer's protocol at a concentration of 5 μmol/L.

Mice-treatment schedule. The C57BL/6 mice were inoculated subcutaneously (s.c.) with MC38 tumor cells (1.2×10^6 in 0.2 ml/mouse). After 14 days, the tumor-bearing mice received the TAg-loaded DCs (JAWS II/EGFP/TAg or BM-DCs/CFDA/TAg) vaccine. Unloaded JAWS II/EGFP and BM-DCs/CFDA cells were used as negative controls. The DC vaccines were injected peritumorally (p.t., 1×10^6 cells/0.2 ml/mouse). On the first, third, fifth, and seventh days after DC injection the mice were killed and the tumors, tumor-draining lymph nodes (tLNs), and spleens from the vaccinated mice were dissected. The tumors and tLN fragments were snap frozen and stored at -70°C prior to cryo-sectioning for immunohistological analysis. The remaining spleen and tLN cells were suspended in PBS without Ca^{2+} and Mg^{2+} (PBS^-) containing 2.5% FCS and used for immune cell subpopulations characteristic studies or frozen for further restimulation and cytotoxicity assays.

Flow cytometric analysis of cell-surface phenotype. For the analysis of cell surface phenotype, isolated spleen and lymph node cells were incubated with the monoclonal antibodies (mAbs) PE anti-CD4, FITC anti-CD8, FITC anti-CD45, or mouse-anti-NK.1.1 and FITC-labeled rat anti-mouse antibodies (all BD Pharmingen). The cells were stained for 45 min at 4°C in a direct test, and for 30 and 45 min in indirect test. The analysis was carried out using a Becton Dickinson FACS Calibur apparatus with Cell Quest Software.

Detection of apoptosis. Apoptosis was detected by the TUNEL (TdT-mediated dUTP nick end labeling) method. Cryostate sections of the tumor tissues were fixed in 4% paraformaldehyde, washed in PBS- for 30 min, permeabilized in a freshly prepared 0.1% Triton-X-100 - 0.1% sodium citrate solution for 2 min at 4°C, and washed (2 × 5 min) with PBS^-. Then the TUNEL reaction mixture containing terminal transferase and FITC-16dUTP (Boehringer Mannheim, Germany) was added to the sample and incubated at 37°C in the dark for 60 min. After incubation, the slides were washed three times with PBS^-, embedded with antifade, and analyzed.

Histological staining. Tumor tissue cryo-sections 5-10 μm thick, were fixed in 4% paraformaldehyde (30 min at 4°C).

Determination of DC migration abilities. Cryosections of lymph nodes and tumors were counterstained with propidium iodide (PI, 2 μg/mL, Sigma) and the presence of green cells, JAWS II/EGFP cells or BM-DCs/CFDA cells, was evaluated in a direct test by fluorescence microscopy.

Hematoxylin and eosin staining: Cryosections of lymph nodes and tumors were stained with hematoxylin and eosin and examined by light microscopy.

Detection of T cells: Cryosections of lymph nodes and tumors were incubated overnight with rat anti-CD4 or rat anti-CD8 antibodies (BD Pharmingen). The slides were then washed with PBS^- and incubated with goat anti-rat antibody conjugated with Alexa Fluor 488 (Molecular Probes). After one-hour incubation, the slides were washed and counterstained with PI (2 μg/mL). All fluorophore-labeled tissue sections were analyzed using a BioRad MRC 1024 scanning confocal fluorescence microscope equipped with LaserSharp software. Cryosections from three distinct parts of tumor from each individual mouse were analyzed.

Cytotoxic assay and determination of IFN-γ and IL-4 production by activated spleen cells. Spleen cells from vaccinated mice were isolated and co-cultured (restimulated) for five days with mitomycin C-treated MC38 cells (50 μg mitomycinC/3×10^6 cells/mL 5% CM incubated 30 min in 37°C) in the presence of IL-2. The source of mouse IL-2 was X63mIL-2 plasmacytoma cell supernantant used at a concentration of 3%, corresponding to 100 U/mL. After five days of restimulation, the culture supernatants were collected and stored at -20°C. The cytotoxicity of the spleen cells was tested by flow cytometry. Target MC38/0 cells were stained with DiOC18 lipophilic dye (Molecular Probes) for 30 min at 37°C and then washed with PBS^-. Spleen cells were incubated after restimu-

Table 1. Efficiency of BM-DCs/CFDA migration from the vicinity of tumor to tumor-draining lymph nodes. Cytometric analysis of the tumor-draining lymph node cell suspension was performed on the 1st, 3rd, 5th, and 7th days after injection of BM-DCs/CFDA cells. BM-DCs/CFDA with green fluorescence were analyzed.

Days after DCs injection / Tested cell groups	1st day		3rd day		5th day		7th day	
	Number of DCs/mln cells	% DCs	Number of DCs/mln cells	% DCs	Number of DCs/mln cells	% DCs	Number of DCs/mln cells	% DCs
BM-DCs/CFDA	40	0.004	26	0.003	72	0.007	22	0.002
BM-DCs/CFDA/TAg	36	0.004	72	0.007	118	0.012	104	0.010

lation with labeled target cells for four hours at 37°C at a 10:1 ratio of effector to target cells. Then the cells were washed and dead cells were stained with PI. Samples were analyzed by flow cytometry on FACS Calibur using FL1 and FL3 filters. The percentage of dead cells (PI-positive) among the $DiOC_{18}$ labeled target cells was calculated.

To measure IFN-γ and IL-4 production by activated splenocytes, commercially available ELISA kits (Pharmingen) were used.

Results

Migratory ability of TAg-loaded DCs from an injection site to tumor-draining lymph nodes

Mouse colon carcinoma-bearing C57BL/6 mice were injected peritumorally (p.t.) with TAg-loaded DCs: BM-DCs stained with CFDA dye (hereinafter BM-DCs/CFDA/TAg) or JAWS II cells transduced with EGFP gene (JAWS II/EGFP/TAg). Unloaded JAWS II/EGFP and BM-DCs/CFDA cells were applied as a negative control. On the first, third, fifth, and seventh days after injection of DCs, the tumors and tumor-draining lymph nodes (tLNs) from each animal group were dissected. The presence of green fluorescent DCs was estimated by fluorescence microscopy (tLN and tumors slides) and flow cytometry (tLN cells). It was observed that DCs injected p.t. migrated to the tLNs (Fig. 1). Furthermore, histological analysis revealed the presence of DCs in tLNs already on the first day after injection. The BM-DC/CFDA number (both loaded and unloaded with TAg) evaluated by flow cytometry increased gradually until the fifth day and then decreased. In addition, the experiments showed that the BM-DCs/CFDA/TAg cells migrated to the tLNs more effectively than did the BM-DCs/CFDA cells ($118/10^6$ and $72/10^6$ total cells, respectively). The numbers and percentages of BM-DCs/CFDA in tLNs are presented in Table 1.

It was also considered whether all the p.t. injected DCs migrated to the tLNs. Most of the DCs remained in the vicinity of the tumor (Fig. 2) and survived at the site of injection until the 7th day after vaccination. This applied especially to JAWS II/EGFP/TAg, but also unloaded JAWS II/EGFP cells. In contrast, after that time only debris of injected BM-DCs was visible in the vicinity of tumor.

Spleen and lymph node cell effector activity after p.t. injection of TAg-loaded DCs

The next step in the investigation was to estimate the percentages of T cells in dissected tLNs and spleens, the cytotoxic activity of tLNs and spleen cells, and their abilities to produce IFN-γ and IL-4 after TAg-restimulation *in vitro*.

The percentage of CD4- and CD8-positive cells did not change in tLNs or in spleens isolated between the 1st- and 7th days after DC injection compared with controls (healthy and tumor-bearing mice untreated with DCs, data not shown).

Interactions between DCs and T cells (CD4 or CD8) were examined by confocal microscopy in tLN cryo-sections labeled with anti-CD4 or anti-CD8 antibodies. Both TAg-loaded and unloaded DCs interacted with CD4 cells already on the 3rd day after DC injection (Fig. 3). Interaction between DCs and CD8 lymphocytes was not clearly visible. Due to the presence of DC-T lymphocyte clusters in the tLN sections, the efficacy of different DC-based vaccines in immune response stimulation was further investigated. No significant differences in the levels of immune response activation were observed between animals which received TAg-loaded or control DCs. In the experiments, the cytotoxicity of restimulated tLN cells gradually increased up to the 5th day after DC injection and then dropped back to the initial level. Only after JAWS II/EGFP cell injection did the cytotoxic activity of the tLN cells stay at the same level up to the 7th day. Regardless of these observations, BM-DCs/CFDA and BM-DCs/CFDA/TAg appeared to be more efficient activators of immune response than TAg-loaded and unloaded JAWS II/EGFP cells. On the 5th day after BM-DCs/CFDA cell injection, the average percentage of tLN cell cytotoxicity reached 43%, whereas after vaccination with BM-DCs/CFDA/TAg cells it reached 39%. Although the cytotoxic activity triggered by BM-DCs/CFDA/TAg injection was lower than that initiat-

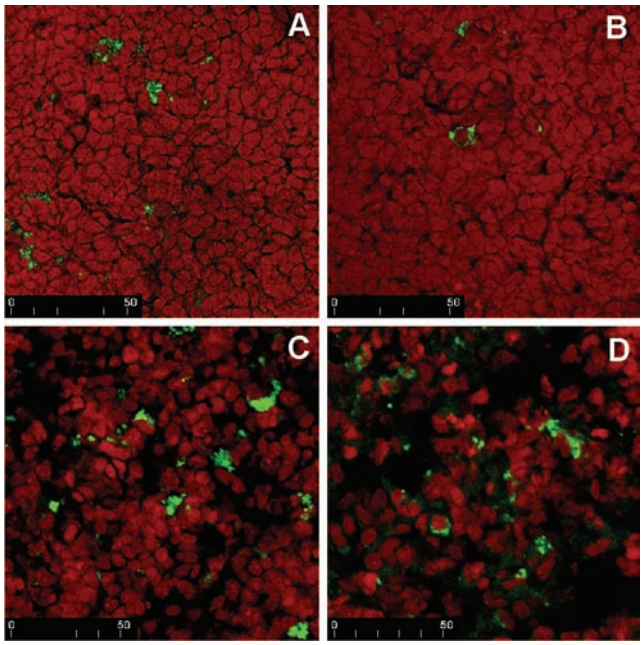

Fig. 1. MC38 tumor-draining lymph nodes 5 days after injection of **A**. JAWS II/EGFP; **B**. JAWS II/EGFP/TAg; **C**. BM-DCs/CFDA; **D**. BM-DCs/CFDA/TAg. Intracytoplasmic green fluorescence of JAWS II/EGFP or BM-DCs/CFDA cells (loaded or unloaded with TAg) and the red nuclei of all the cells located in lymph node (stained with PI 2 μg/ml) are visible.

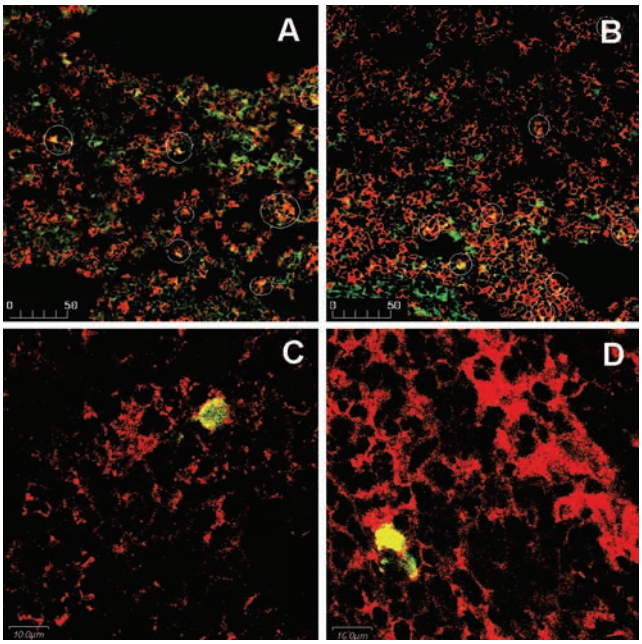

Fig. 3. Visualization of DC and CD4+ T-cell interactions. Tumor-draining lymph node tissue sections were stained with anti-CD4 conjugated with Alexa fluor 546 (red). In the pictures are visible the interaction of CD4 cells with DCs (green): **A**. JAWS II/EGFP cells; **B**. JAWS II/EGFP/TAg cells; **C**. BM-DCs/CFDA cells and **D**. BM-DCs/CFDA/TAg cells. All on the 3rd day after DCs injection.

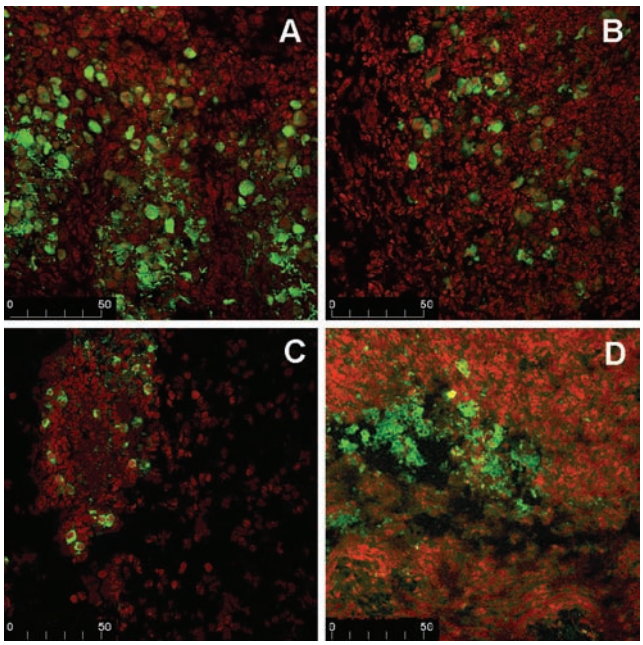

Fig. 2. Tumor tissue after p.t. injection of DCs-based vaccine. Intracytoplasmic green fluorescence of JAWS II/EGFP or BM-DCs/CFDA cells (loaded or unloaded with TAg) and also the red nuclei of surrounding cells (stained with PI 2 μg/ml) are visible. In the pictures the edge of the tumor tissue on the 7th day after injection of **A**. JAWS II/EGFP; **B**. JAWS II/EGFP/TAg cells; **C**. BM-DCs/CFDA/TAg cells; and the 3rd day after injection **D**. BM-DCs/CFDA cells.

ed by BM-DCs/CFDA injection, it decreased at a slower rate (amounting to 29% and 25% on the 7th day, respectively). Unexpectedly, vaccines consisting of JAWS II/EGFP or JAWS II/EGFP/TAg cells resulted in significantly lower tLN cell cytotoxicity (23%) compared with both types of BM-DC/CFDA cells. Furthermore, injection of these cells evoked an inconsiderable increase of tLN cell cytotoxicity over controls (20% for untreated mice and 13.5% for healthy mice). JAWS II/EGFP/TAg cells proved to be slightly better activators of the immune response than JAWS II cells (Fig. 4a).

The cytotoxicity of spleen cells was lower than that of tLN cells (reaching a maximum of 25%), but their tendency to change was similar to that observed in tLN cells (Fig 4b). Vaccination with BM-DCs or JAWS II cells resulted in only limited differences in the cytotoxicity of spleen cells and was not as distinct as that observed in tLN cells.

Generally, cytotoxic activity was accompanied by the immune cells' ability to produce IFN-γ (Fig 5a, 5b). The maximum IFN-γ level (average: 40 ng/mL) was detected in supernatants collected from cultures of restimulated tLN cells which were harvested on the 5th day after BM-DCs/CFDA cell vaccination. The production of IL-4 was slight and that of TNF-α was undetectable.

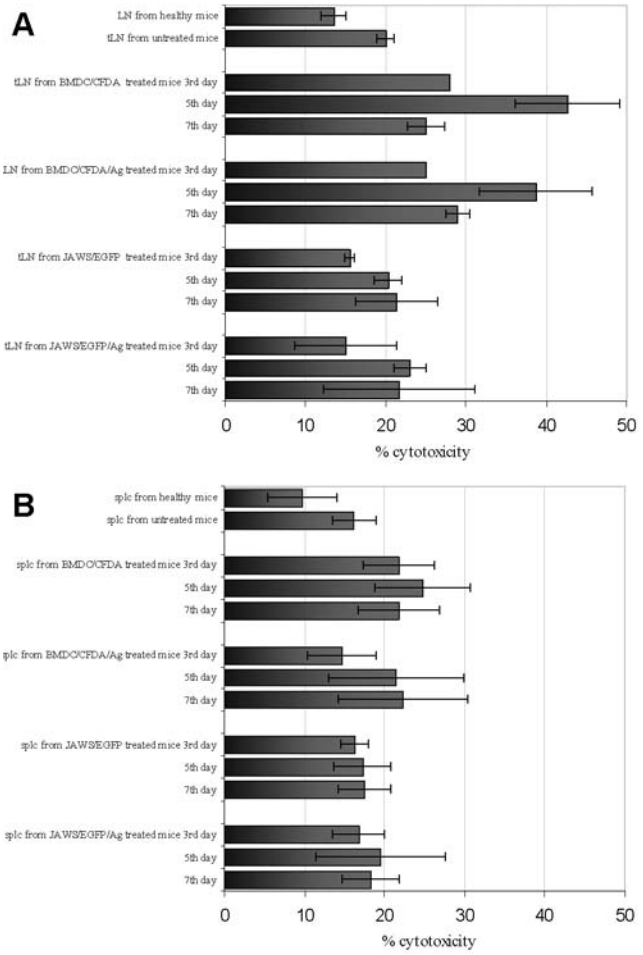

Fig. 4. Cytotoxic activity of tLN (**A**) and spleen (**B; splc**) cells collected from mice on the 3rd, 5th, and 7th days after injection of DCs. The cells were restimulated *in vitro* with MC38 cells and then the cytotoxic assay was performed by flow cytometry. To calculate the mean and standard deviation three mice from each presented group were tested. % cyotoxicity - the percentage of dead target MC38 cells.

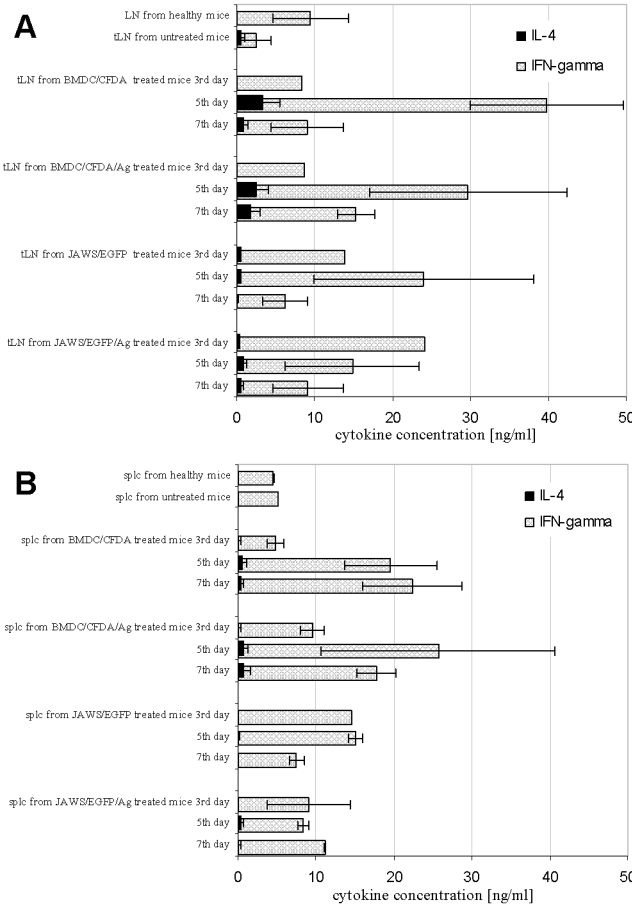

Fig. 5. Production of IFN-γ and IL-4 by restimulated *in vitro* tLN (**A**) and spleen (**B**) cells. The concentration of IFN-γ and IL-4 was measured by ELISA in supernatants collected from the above restimulated tumor-draining lymph node (tLN) or spleen (splc) cells after DC injection. To calculate the mean and standard deviation three mice from each presented group were tested.

Histological and immunohistological analysis of immune cells infiltrating tumor tissue

Analyses of lymphocyte influx into the connective tissue surrounding the tumor tissue and the level of tumor cell apoptosis were performed on the 7th day after DC injection. Histological examination of 14-day-old tumors (when nodules were already palpable under the skin) revealed that they were compact and surrounded by connective tissue nodules. In the tumor tissue the closely adjacent tumor cells with well-developed connective tissue crossing the whole tumor tissue were present. There were also a few infiltrating CD8 and CD4 cells in the connective tissue, but not between tumor cells. Seven days after injection of BM-DCs/CFDA/TAg and JAWS II/EGFP/TAg cells, numerous necrotic areas and a few apoptotic bodies appeared in the whole tumor tissue (Fig. 6b, c). This observation was confirmed by the more specific TUNEL technique (detection of characteristic DNA fragmentation, Fig. 6 d-g). In addition, the infiltration level of tumor tissue by CD4 and CD8 lymphocytes was similar to that observed in control tumor tissue and was stable (data not shown). On the other hand, vaccination with BM-DCs/CFDA or JAWS II/EGFP cells as well as with physiological saline did not result in damage of the tumor tissue (Fig. 6a).

Discussion

The main function of DCs is antigen uptake and its further presentation to immune effector cells. During the process, DCs leave an inflammation site and migrate through lymphatic vessels to lymph nodes. During migration the DCs change their morphological and functional features and reach maturity, at which time they can act as efficient activators of naive T cells. In reply to antigen presentation and IL-12 production by

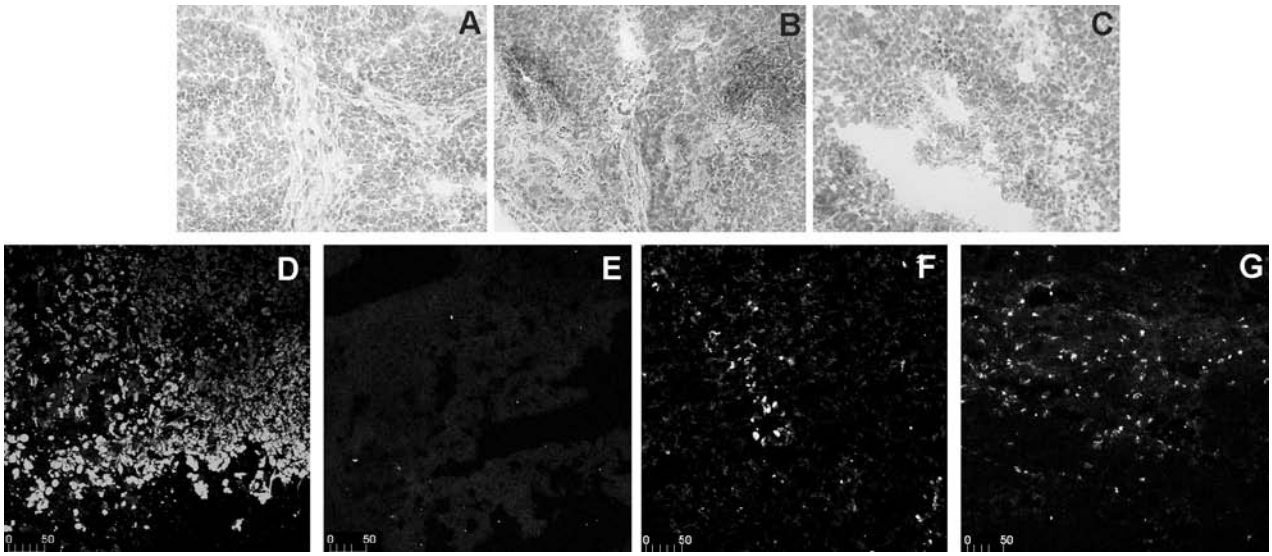

Fig. 6. Apoptotic and necrotic cells in MC38 tumor tissue. Tumors were collected on the 7th day after DC injection. The tissue sections were stained with H+E to analyze changes in tumor tissue structure (A-C) or stained with TUNEL reaction mixture containing terminal transferaze and FITC-16dUTP to identify apoptotic cells (D-F). **A**. tumor tissue (TT) from untreated mouse (magnification × 400). In the picture closely adjacent tumor cells with well-developed connective tissue containing fibroblasts are present; **B**. TT after injection of JAWS II/EGFP/TAg cells (magnification × 400); **C**. TT after injection of BM-DCs/CFDA/TAg cells (magnification × 400); B-C: in the whole tumor tissue, numerous necrotic areas and few apoptotic bodies are visible. Identification of apoptotic cells: **D**. positive control; **E**. negative control; **F**. apoptotic cells in tumor tissue after injection of JAWS II/EGFP/TAg cells; **G**: apoptotic cells in tumor tissue after injection of BM-DCs/CFDA/TAg cells.

DCs, T cells acquire the ability for clonal expansion and migration from lymph nodes to an inflammatory site [1,2].

Detailed analyses of the migration of TAg-loaded DCs, injected *p.t.* to MC38 colon carcinoma-bearing mice, and their ability to activate a specific immune response were performed in this study. In the experiments, two types of murine DCs were applied: bone marrow-derived DCs (BM-DCs), maintained *ex vivo*, and cells of the JAWS II line, possessing features of immature myeloid DCs. All cells were loaded with antigen prepared from MC38/0 cell lysate (TAg). We chose stimulating DCs with lysate because according to generally accepted information, loading DCs with whole tumor cell components should generate a T-cell response (both $CD4^+$ helper T cells and $CD8^+$ CTL) to tumor-associated antigens. In consequence, this may reduce the possibility of a tumor escaping the host immune response [9,10].

Our findings demonstrate that BM-DCs/CFDA cells or JAWS II/EGFP cells (TAg-loaded as well as unloaded) injected p.t. were able to migrate to tumor-draining lymph nodes. Both histological and cytometric analysis revealed that these DCs appeared in tLNs already on the first day after injection, reaching a maximum on the 5th day. Lapin [11] and Martin-Fontecha [12] examined the number of DCs infiltrating mouse popliteal LNs after DC injection into the footpad. Maximal numbers of DCs were detected in the lymph nodes between the second and fourth day after DC injection. In contrast, when DCs were injected s.c. they rapidly infiltrated the draining LN, achieving a maximum within 30 min and remained in the node for several hours [13]. The observed differences in the kinetics of DC migration might be caused by the different ways of DC injection. Therefore we suggest that p.t. injection results in an initial sequestration and slower efflux of DCs from an injection site to draining lymph nodes. Moreover, immunosuppressive factors such as IL-10, TGF-β, VEGF, or PGE2, often produced by growing tumors, could have an additional negative impact on DCs' migratory efficacy [14]. Consequently, part of the injected DCs could be forced to remain in the vicinity of the tumor (as observed in our experiments). On the other hand, TAg-loaded DCs remaining in the vicinity of tumor could be able to change a hostile tumor environment and trigger a more efficient anti-tumor response.

Our observations also demonstrate that TAg-loaded BM-DCs migrated to tLNs more effectively than unloaded BM-DCs. Nevertheless, the number of the cells in tLNs never exceeded 0.012%. Similar results were obtained in experiments performed by Lappin *et al.* [11]. Moreover, they observed the an increased retention of DCs in LNs with increased number of injected cells. However, Martin-Fontecha *et al.* [12] noted that the number of infiltrating DCs reached a plateau when the number of injected DCs was 2×10^6.

They suggest that the T-cell area of the LN may accept only a limited number of exogenous cells. Alternatively, DC infiltration may be limited by saturation of the lymphatic vessels through which DCs migrate into the T-cell areas of the LN. In our experiments, DC vaccines containing ca. 1×10^6 of cells were applied; therefore we supposed that the number of DCs retained in tLNs could have been higher if we had injected a higher number of cells.

The next stage in our investigation was to evaluate the possible interactions between DCs and T lymphocytes. It is well-known that exogenous antigens can enter the MHC class I presentation pathway of APCs and thereby prime CD8 T cells in a process generally termed "cross-presentation" [15,16]. However, in our experiments, DCs (TAg-loaded, but also unloaded with TAg) made clusters with CD4 T cells, but interactions between DCs and CD8 T cells were not clearly visible. Because of this observation, spleen and tLN cell effector activities as well as tumor tissue infiltration by immune cells were examined. BM-DCs/CFDA cells, both loaded and unloaded with TAg, proved to be more efficient immune response activators than JAWS II/EGFP cells. They caused increased cytotoxicity of spleen and lymph node cells and the ability to produce IFN-γ compared with controls. Despite the lack of differences in the cytotoxic activities of tLN or spleen cells obtained from mice vaccinated with TAg-loaded and unloaded DCs, changes in tumor tissue structure were observed only after injection of TAg-loaded DCs and only seven days after vaccination.

In conclusion, TAg-loaded DCs migrated more efficiently to tLNs, and were more effective activators of local (but not systemic) cellular immune response compared with unloaded DCs. No significant differences between the effector activities of spleen and tLN cells, regardless of the type of vaccine applied, were observed. This presumably resulted from the way of injection, because DCs injected in the vicinity of a growing tumor had direct access to tumor antigen and thus the host response evoked by both TAg-loaded and unloaded DCs could be similar. We hypothesize that only the application of TAg-loaded DCs to tumor bearing mice as an adjuvant supporting chemotherapy may activate a more effective anti-tumor response.

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