Vaccine 28 (2010) 8162–8168

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Vaccination with dendritic cells charged with apoptotic/necrotic B16 melanoma induces the formation of subcutaneous lymphoid tissue

Soledad Mac Keon[a,b], Silvina Gazzaniga[a], Julieta Mallerman[a], Alicia I. Bravo[c], José Mordoh[b], Rosa Wainstok[a,b,*]

[a] Depto. de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina
[b] Fundación Instituto Leloir-IIBBA CONICET, Buenos Aires, Argentina
[c] Hospital Eva Perón, San Martín, Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 18 August 2010
Received in revised form
18 September 2010
Accepted 25 September 2010
Available online 27 October 2010

Keywords:
Vaccination
Melanoma
Dendritic cell
Apoptotic cell
Lymphoid tissue

ABSTRACT

Antigen presentation by dendritic cells (DC) is of key importance for the initiation of the primary immune response. Mice vaccinated with DC charged with apoptotic/necrotic B16 cells (DC-Apo/Nec) are protected against B16 challenge. The aim of this study was to assess vaccine cell migration in our system and to find out if there is an immunological response taking place at the vaccination site. The formation of a pseudocapsule, peripheral node addresin expression in small venules, and the recruitment of a wide variety of cellular populations, including macrophages, polymorphonuclear lymphocytes, and CD8+ and CD4+ T lymphocytes found in association with DC, evidenced the formation of tertiary lymphoid tissue in the vaccination site in our experimental system.

1. Introduction

Melanoma is an immunogenic cancer and many strategies have employed dendritic cells (DC) to enhance specific immunity in preclinical models and in clinical studies [1–4]. Antigen presentation by DC is of key importance for the initiation of the primary immune response [5,6]. This special capacity derives from their ability to take up, process and transport antigens (Ags) from the periphery to draining lymphoid organs, which support induction of T cell responses [7,8]. After Ag capture in the periphery, Ag-presenting cells (APC) migrate via afferent lymphatic vessels to secondary lymphoid tissues while naive T cells enter the lymph nodes through specialized blood vessels such as high-endothelial venules (HEV). Lymph nodes provide the suitable cytokine milieu and specialized structures to attract both APC and naive T cells, orchestrate their localization within the lymph node and thereby enable their interaction [9]. Several lines of evidence indicate a relevant contribution of DC to induce and maintain secondary lymphatic tissues [10].

A broad effector and memory immune response dependent on CD4+ and CD8+ T cells can be induced against tumor Ags when apoptotic/necrotic (Apo/Nec) tumor cells are presented by DC [11]. Our vaccination scheme, which consists of 4 s.c. injections of 2×10^5 DC charged with B16-F1 Apo/Nec melanoma cells (DC-Apo/Nec), generates 80% protection against tumor challenge [11]. When 2×10^5 DC-Apo/Nec were administered s.c. in the footpad only about 0.5% of the cells composing the vaccine migrated to draining lymph nodes [11]. This percentage is similar to that obtained when LPS-matured DC were injected in the footpad, the efficiency of mature DC migration being dose-dependent [12,13]. In spite of this low migration, a potent immune response and protection is triggered. This suggests that although the cells composing the DC-Apo/Nec vaccine fail to reach secondary lymphoid tissue, they exert a strong effect on the immune system.

The aim of this work was to assess DC migration in our vaccination model, in which the vaccine is administered s.c. in the inguinal region. We demonstrate in this paper the *de novo* formation of s.c. lymphoid tissue at the vaccination site and suggest its importance for immune protection.

2. Materials and methods

2.1. Animals and cell lines

8–10 weeks old male C57BL/6 (HH-2^b) mice were obtained from the Facultad de Ciencias Veterinarias, University of La Plata

* Corresponding author at: Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Cuidad Universitaria, Pabellón II, 4to piso, C1428EGA Buenos Aires, Argentina. Tel.: +54 1145763342.
E-mail address: rwains@qb.fcen.uba.ar (R. Wainstok).

0264-410X/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.vaccine.2010.09.095

(Buenos Aires, Argentina). Mice were maintained in pathogen-free conditions, and studies were performed in accordance with local ethical guidelines. The syngeneic melanoma B16-F1 line (gift from Dr. A. Vecchi. Instituto Mario Negri, Milan, Italy) was utilized, and was maintained in culture in DMEM (Sigma–Aldrich, MO) supplemented with 10% heat-inactivated FBS (Natocor, Córdoba, Argentina), 100 U/ml penicillin and 100 μg/ml streptomycin (Life technologies, Gaithersburg, MD). To detach B16-F1 cells from the flask, PBS and 2 mM EDTA were used (5 min incubation at 37 °C). The line was periodically tested to be mycoplasm-free.

2.2. Induction of apoptosis and necrosis

B16-F1 cells were resuspended in DMEM for irradiation (70 Gy, Siemens lineal accelerator, Instituto Alexander Fleming, Buenos Aires, Argentina). Irradiated cells were stored in liquid nitrogen until use. 2 days before coculture with the DC, irradiated cells were thawed, washed twice, cultured for 48 h and the non-adherent were harvested by pipetting. The adherent cells were detached with EDTA.

2.3. Culture of bone marrow derived DC and coculture with apoptotic cells to generate the DC-Apo vaccine

Bone marrow cells were harvested from femurs and tibias of normal C57BL/6 mice and washed with PBS. Cells were resuspended in RPMI 1640 (Sigma–Aldrich, MO) supplemented with 10% inactivated FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.02 μg/ml murine GM-CSF (Preprotech, Mexico DF, Mexico). On day 3 fresh medium was added. On day 7, DC were harvested by gentle pipetting, and cocultured in the DC conditioned medium with Apo/Nec B16 cells in a 1/1 ratio for 24 h. For vaccination use, DC were washed twice with PBS.

Routinely, as previously reported [11], DC and maturation markers were monitored before and after coculture. The expression of CD11c remained similar while CD80, CD86 and MHC II were upregulated in tumor exposed DC.

2.4. Vaccination of mice

2×10^5 DC-Apo/Nec or vehicle (PBS) were injected s.c. once a week, for 4 weeks. Mice were vaccinated in three ways: (a) in the inguinal region, alternating both flanks, (b) in the inguinal region, always on the same flank, and (c) alternating the homolateral axillar and inguinal region of the animals. The B16 challenge (1.3×10^4 viable cells) was administered on the contralateral flank to the last vaccine. Animals were monitored for tumor growth by palpation, and tumor size was measured with a Vernier caliper. The animals were killed when tumors displayed a size of 400 mm^3.

2.5. In vivo migration of the cells composing the DC-Apo/Nec vaccine

When fluorescent marking of the vaccine was required, on day 7, previous to the coculture, DC were dyed green with 25 mM PKH-67 (Sigma–Aldrich, MO) and Apo/Nec were dyed red with 25 mM PKH-26 (Sigma–Aldrich, MO) according to the manufacturer's instructions. Mice were injected in the inguinal flank s.c with 2×10^5 stained DC-Apo/Nec, and after 24 h the presence of stained cells in the site of injection and in draining lymph nodes (popliteal, inguinal and axillary lymph nodes) was detected by flow cytometry (BD FACS Calibur, Instituto Alexander Fleming, Buenos Aires, Argentina). Events were analyzed using WinMDI2.8 software. To prepare single-cell suspensions from the flank tissue, hair was removed with a razor, and the site of interest cut out (removing first the inguinal lymph node). It was placed in a plate in RPMI 2 μg/ml collagenase IA at 4 °C (Sigma–Aldrich, MO), where it was mechanically disaggregated. An equal volume of DNAse I (Invitrogen, Carlsbad, CA) was added and it was incubated at 37 °C for 30 min, vortexing every 10 min. The cells were pelleted at 1100 rpm for 7 min, resuspended in EDTA 2 mM, and incubated for 15 min at 37 °C. Cells were then pelleted at 1100 rpm for 7 min, resuspended in fresh RPMI, and a 1 ml syringe, drawn up and down, was used to continue disaggregating. The cell suspension was then put through a 40 μm pore-size nylon, and then the cells were pelleted at 1100 rpm for 10 min and resuspended in paraformaldehyde 1%. To prepare single-cell suspensions from the draining lymph nodes the same protocol was followed, with the exclusion of the incubation with EDTA.

To assess DC-Apo/Nec biodistribution utilizing the IVIS Lumina bioluminometer (Caliper Life Sciences, Hopkinton, MA) the cells were stained with DiR (1 μg/ml) (Invitrogen, Carlsbad, CA) before being administered. Their distribution was tracked non-invasively 1, 5 and 9 days post-vaccination. The injection site and draining lymph nodes of animals killed at day 9 were exposed to the bioluminometer.

2.6. Confocal fluorescence microscopy

The vaccinated sites were stored at −80 °C. The tissues were later fixed with acetone and sectioned with cryostat. To perform the specific fluorescent staining, the sections were hydrated 15 min in PBS, and unspecific binding sites were blocked by incubating 1 h with PBS 10% goat serum. Then sections were incubated with the primary antibodies for 2 h, washed 3 times with PBS, and incubated 2 h with the secondary antibody, when required. All the primary antibodies were from BD (Franklin Lakes, NJ) (5 ng/μl), while the secondary antibody, goat anti-rat cy5 conjugated IgG was from Jackson (West Grove, PA) (7.5 ng/μl). Sections were analyzed using the Zeiss LSM pascal 5 confocal microscope (Fundación Instituto Leloir, Buenos Aires, Argentina).

2.7. Inmunohistochemical staining

For hematoxylin–eosin and PNAd staining animals were killed at different times and the vaccination associated tissue was obtained, fixed in 10% buffered formalin, embedded in paraffin, sectioned at 4–7 μm. For PNAd staining unmasking of antigens was achieved by a 15 min incubation at 95 °C in 10 mM pH 6 citrate buffer. They were washed twice with PBS and unspecific binding sites were blocked by incubating 30 min with 10% goat serum in PBS. Then sections were incubated overnight at 4 °C with the primary antibody (rat anti-mouse PNAd, BD), and then the endogenous peroxidase was inactivated with a 30 min incubation in 4% H_2O_2 in PBS. The sections were washed twice with PBS and then incubated again with the primary antibody 1 h at room temperature. After washing twice with PBS, the sections were incubated 2 h at room temperature with the secondary antibody (anti-rat Peroxidase, Jackson). After washing twice with PBS, they were incubated with DAB (0.7 mg/ml DAB; 0.13% H_2O_2 100 vol) in PBS. The reaction was stopped with distilled H_2O, washed with PBS and counterstained with hematoxylin.

3. Results

3.1. DC loaded with Apo/Nec B16 cells accumulate in the vaccination site

To assess DC migration in our system, we co-cultured dyed red irradiated B16 cells (Apo/Nec) with dyed green immature DC, as described under methods. 24 h after 1 dose of DC-Apo/Nec vaccine, the draining lymph nodes were dissected, and the

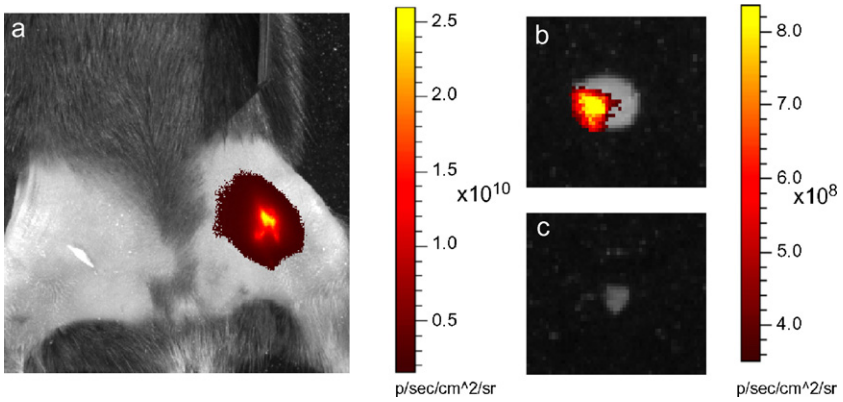

Fig. 1. Visualization of CD-Apo/Nec dyed with DiR. 9 days post-injection the animals were killed and the photon counts emitted by the site of injection (a), the inguinal lymph node (b) and the popliteal lymph node (c) were measured. The scale to the right of each picture describes the color map for the photon count.

number of injected cells which migrated to those nodes was analyzed by flow cytometry, corresponding to 0.8% of the injected cells (data not shown). To study in more detail the homing pattern of the DC-Apo/Nec cells, they were dyed with DiR (-1,1′-dioctadecyl-3,3,3′,3′-tetramethylindotricarbocyanine iodide), and their distribution was tracked non-invasively by *in vivo* imaging. The injection site (in the inguinal region) and draining lymph nodes of animals killed at day 9 post-vaccination were exposed to the bioluminometer. After injecting one dose of labeled DC-Apo/Nec cells, 95% of the cells remained at the injection site up to 9 days post-vaccination (Fig. 1a). At day 9 after vaccination, 5% of dyed material reached the lymph node, while no dyed cells were detected in the popliteal lymph node of the same flank (Fig. 1b and c). Since DC-Apo/Nec cells accumulated mostly in the vaccination site, and in our previous vaccination model the vaccine was applied on both flanks of the animals, coinciding the B16 challenge site with one of the vaccination sites, we questioned whether the lack of tumor growth obtained was due to a regional rather than to a systemic protection.

3.2. Immunization with 4 doses of DC-Apo/Nec vaccine results in long-lived systemic protective immunity

In the previous vaccination scheme [11] vaccinations were performed in alternated flanks of the animal, and the tumor challenge was performed in one of both flanks. To discard the possibility that the B16 viable cells were rejected by a local pre-existing inflammation, mice were vaccinated on one flank and challenged on the contralateral flank. Two approaches were utilized. A group of mice was given the 4 doses on the same inguinal region, and received the tumoral challenge on the contralateral flank (Group 1). A second group received the 4 doses on the same flank, alternating between the homolateral inguinal and axillary regions, with the challenge performed on the opposite flank (Group 2). In both groups 90% of the animals remained tumor-free 10 weeks after being challenged, compared to 10% in the control group (PBS) (Fig. 2). This difference was statistically significant (Logrank Test $p < 0.01$). Therefore, the lack of tumor growth previously reported is in fact due to systemic protection and not to a local effect.

3.3. Tertiary lymphoid tissue detected after vaccination with DC-Apo/Nec

It was not clear how the DC-Apo/Nec vaccine, failing to migrate to the lymph nodes, nonetheless exerted a great effect on the generation of long lasting systemic anti-tumor protection. To characterize how DC-Apo/Nec interacted locally with the host immune system to elicit resistance to tumor growth, the site of injection was analyzed. Skin sections obtained 5 days after completion of the vaccination scheme were compared with several controls: DC, Apo/Nec, DC plus Apo/Nec without previous coculture (DC+Apo/Nec), and vehicle (PBS).

An inflammatory reaction was observed in the DC-Apo/Nec injection site, organized as a definite structure showing a framework of reticulated fibers and pseudocapsulae with multiple crossing fibroblastic trabeculae, similar to a lymph node (Fig. 3a and b). Cells present in this structure were mainly polymorphonuclear leukocytes, lymphocytes, macrophage-type cells and giant cells (Fig. 3c). With Apo/Nec cells (Fig. 3d and e), DC, DC+Apo/Nec, or PBS administration it was not possible to find this structure. Scarce recruitment of PMN leukocytes, macrophages and lymphocytes was noted when 4 doses of DC or DC+Apo/Nec were administered (data not shown). The infiltration was less important after the administration of Apo/Nec cells alone (Fig. 3f).

3.4. CD8+ and CD4+ T lymphocytes are recruited to DC rich areas in the vaccination site

To evaluate in detail the populations present in the tertiary lymphoid tissue formed at the site of DC-Apo/Nec administration, inmunofluorescence and confocal microscopy analysis were performed.

The presence of cells that stained positively for both the DC marker CD11c and the macrophage marker F4/80 could be observed by confocal microscopy 24 h post-completion of the vaccination scheme, as well as a single positive F4/80 cell accumulation

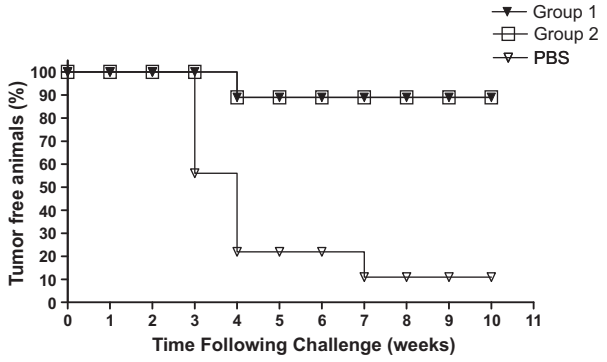

Fig. 2. Long-lived systemic protective immunity after 4 doses of CD-Apo/Nec. Group 1: animals received 4 doses of CD-Apo/Nec on the inguinal region of the same flank, and the tumoral challenge on the contralateral flank. Group 2: animals received alternated doses between the inguinal and axilar regions of the same flank, while the challenge was performed on the opposite flank. PBS: received 4 PBS injections and a tumoral challenge. Logrank test; $p < 0.01$ for Group 1 and Group 2 vs PBS.

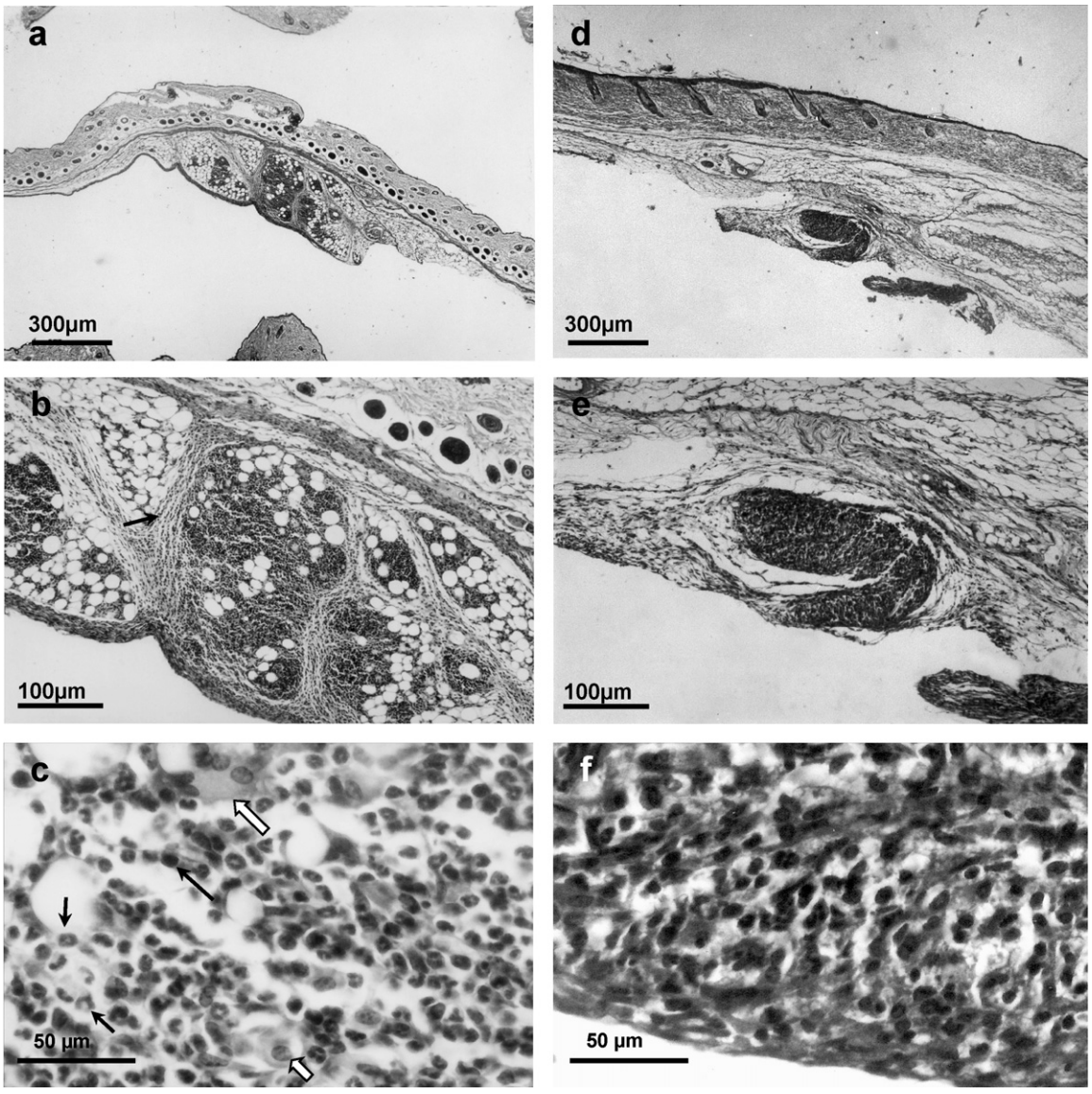

Fig. 3. Pseudo-lymphoid structure detected 5 days after vaccination with DC-Apo/Nec. Sections of the vaccination sites were obtained and hematoxylin–eosin stained ($n = 10$ for each group). (a–c) DC-Apo/Nec vaccination, with increasing magnifications. (d–f) Apo/Nec vaccination, with increasing magnifications. (a) Section of vaccination site with intense cellular infiltration and irregular borders. (b) A definite structure showing a framework of reticulin fibers and pseudocapsule (black arrow) with multiple fibroblastic trabeculae across it. (c) The infiltrate consists of polymorphonuclear leukocytes (small black arrow), lymphocytes (long black arrow), macrophages-type cells (small white arrow) and giant cells (long white arrow). (d) Section of vaccination site with well limited borders. (e) No tertiary lymphoid structure was observed. (f) The cellularity consists in apoptotic tumoral cells and discrete lymphocytic infiltrate.

(Fig. 4a). Polymorphonuclear (PMN) leukocytes (Ly6G+) could also be observed infiltrating the site of injection. Total numbers of CD4+ and CD8+ T cells and DC recruited to the vaccination site were measured by flow cytometry. Cell counts were significantly increased in the vaccination sites as compared to the control groups ($p < 0.05$ for $CD3^+CD8^+$ and $CD3^+CD4^+$; $p < 0.01$ for CD11c+). CD8+ T cells, CD4+ T cells and CD11c+ cells increased 52-fold, 16-fold and 260-fold respectively compared to each control group (Fig. 4b). CD11c+F4/80+ cells, PMN cells, and CD4+ and CD8+ T cells were still present 5 days post-completion of the vaccination scheme (Fig. 4c). These lymphocytic populations were found in close association with DC (Fig. 4d).

3.5. Development of high-endothelial venules (HEV) in the vaccination site

To determine whether lymphocytic homing was recruited to the ectopic lymphoid tissues through HEV as in lymph nodes, the vaccination site was stained for peripheral node addressin (PNAd), a marker for HEV, 5 days after the 4th dose of vaccine. PNAd expression was detected on the luminal surface of veins and cytoplasm of endothelial cells in the injection site, but not in venules or small arteries in the periphery and remote areas of the same skin section or control skin sections (Fig. 5).

4. Discussion

Diverse anti-tumoral strategies rely on *ex vivo* generated autologous DC vaccination to elicit immunological responses, though the scheme for optimal vaccination remains yet to be established. After DC-Apo/Nec vaccination, we observed that only a small percentage of DC migrated to the draining lymph nodes 9 days post-injection, while the majority of the cells remained at the vaccination site. A similar phenomenon has been observed in mice with *ex vivo* derived DC vaccines [14] and in clinical studies where patients were immunized with Ag-loaded DC. In this study we have demonstrated

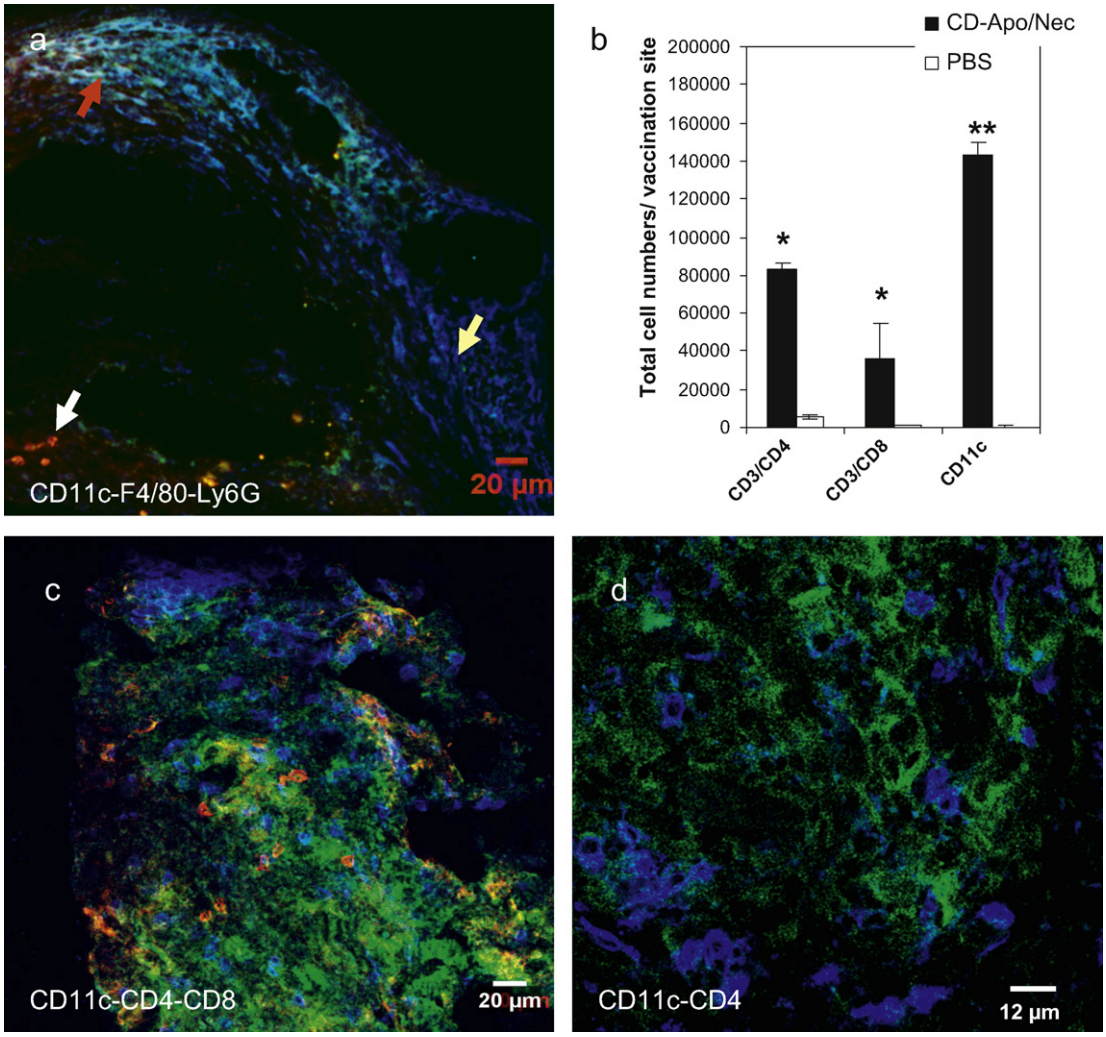

Fig. 4. Lymphocyte recruitment at the vaccination site after completion of the vaccination schedule. (a and b) 24 h post-completion of the vaccination scheme. (a) Confocal microscopy analysis. CD11c+ cells (green), F4/80+ cells (blue), and Ly6G+ cells (red). White arrow showing Ly6G+ cell infiltration, yellow arrow pointing F4/80+ cell infiltration and red arrow pointing the accumulation of double-staining F4/80+/CD11c+ cells (light blue). (b) FACS analysis. Total cell numbers of CD3+CD8+, CD3+CD4+ and CD11c+ populations. Each value represents the mean ± SD of five animals. $^{*}p < 0.05$ for CD3+CD8+ and CD3+CD4+; $^{**}p < 0.01$ for CD11c+, compared with controls (Student's *t*-test). (c and d) Confocal microscopy 5 days post-completion of the vaccination scheme. T CD8+ cells (red) T CD4+ cells (blue) and CD11c+ cells (green).

that after DC-Apo/Nec vaccination a tertiary lymphoid tissue was formed at the vaccination site, and we propose that tumoral rejection would be partially due to this neoformation. The protection was not broken when the vaccination site was heterolateral with the tumor challenge site, thus confirming that tumor rejection was not determined by a local effect.

The characterization of the vaccination site demonstrated the recruitment of a wide variety of cellular populations and the establishment of tertiary lymphoid tissue, evidenced by the formation of a pseudocapsule and PNAd expression in small venules. Inflammatory populations like PMN leukocytes and macrophages were already present 24 h post-completion of the vaccination scheme. DC were clearly visible at the vaccination site, and the presence of F4/80+/CD11c+ cells could be observed 5 days after vaccination. By flow cytometry we determined that 9% of the DC in the DC-Apo/Nec vaccine expresses the macrophage marker F4/80 (data not shown), so the *ex vivo* generated DC could account for part of this double-staining population. CD4+ and CD8+ lymphocytes could be observed at the vaccination site 5 days after completion of the vaccination scheme, when PNAd expression was evidenced in venules. PNAd expressed in HEV of lymph nodes interacts with L-selectin expressed in lymphocytes, mediating their entry to the lymph node parenchyma [15]. Therefore, PNAd expression in venules at the injection site, as well as CD4+ and CD8+ recruitment to DC rich areas, denote a lymph node-like microenvironment. This tertiary lymphoid tissue formation was not evidenced with Apo/Nec, DC, or DC+Apo/Nec vaccination, which do not generate tumoral protection, so this novel structure could be crucial to generate the strong immunostimulatory effect needed for anti-tumoral protection.

The lymphoid neoformation found depended on a 24 h coculture between DC and Apo/Nec tumoral cells. This would evidence the importance of a proper antigenic processing and DC maturation to trigger the formation of the tertiary lymphoid tissue in our experimental system.

It should be taken into account that a large proportion of Apo/Nec cells remain at the injection site, constituting a continuous source of tumor Ag. The induction of a tertiary lymphoid tissue at the tumor site by lymphotoxin-α targeting generates local specific T-cell responses and B16 melanoma destruction, independent of secondary lymphoid tissue [16]. Priming of naive T cells at the tumor site would facilitate access to tumor-derived Ags, which may increase T cell clone diversity and boost the immunological response [17].

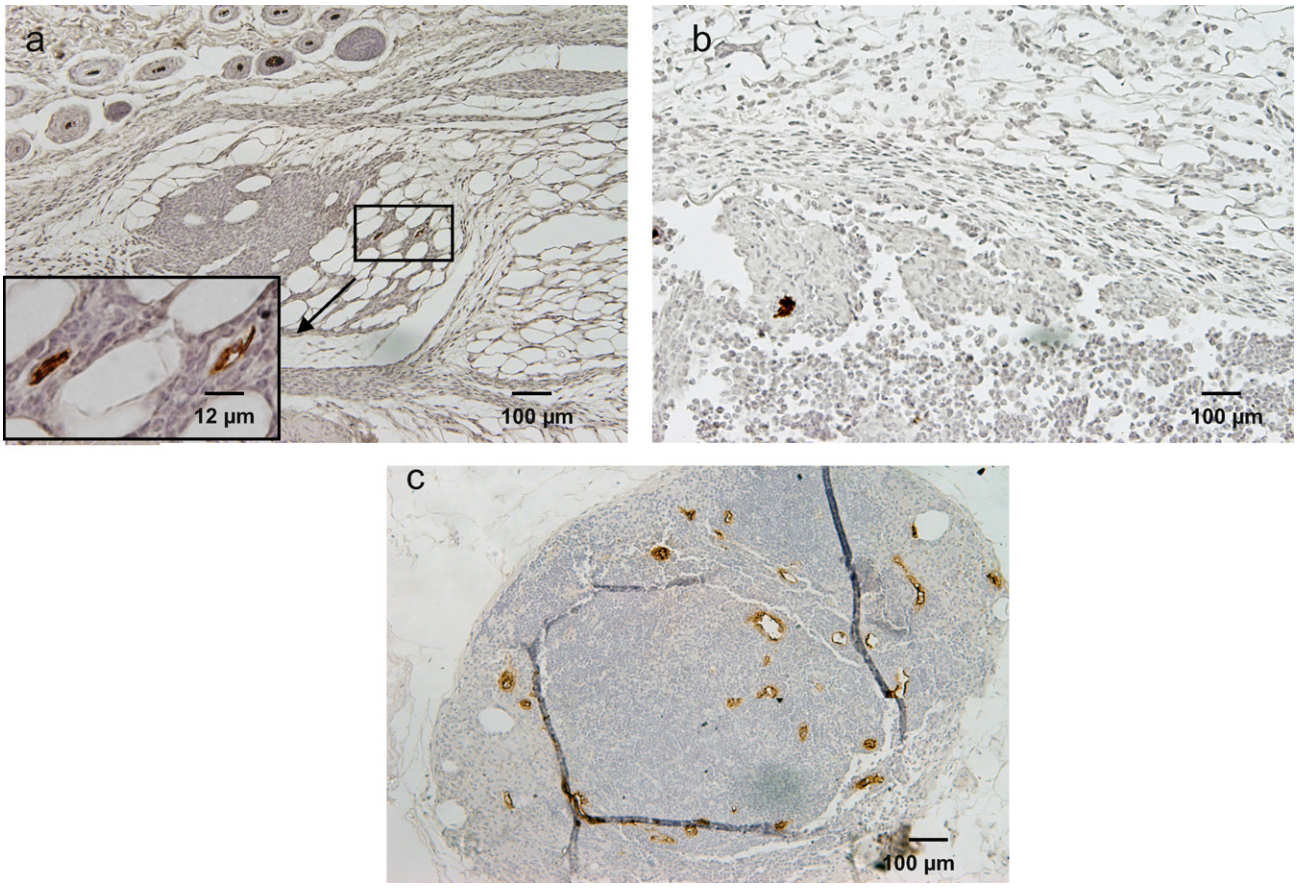

Fig. 5. PNAd expression in the injection site. (a) Skin section of the vaccination site 5 days post-completion of the vaccination scheme, showing PNAd positive staining (brown) on luminal surface of veins and cytoplasm of endothelial cells. (b) Skin section of the vaccination site 24 h after one dose of DCApo/Nec. (c) Positive staining in a control lymph node.

Under conditions of chronic inflammation, accumulation of lymphoid cells as tertiary lymphoid tissues may arise in sites that are normally devoid of canonical lymphoid organs settings [18]. They share several key morphological characteristics with secondary lymphoid organs, such as the presence of HEV, discrete T and B cell accumulations, CD11c+ DC, and follicular DC networks [19]. Tertiary lymphoid tissues may facilitate the interaction between lymphocytes and APC, accelerating the kinetics of immune response. We speculate that the repetitive injection with DC-Apo/Nec induces chronic inflammation, mediating an immune response that may sustain or exacerbate disease in autoimmune diseases [17,20] or induce rejection in allograft or organ transplantation [21], but that could be beneficial when generating anti-tumoral protection.

There are evidences on the crucial role of DC in the formation of tertiary lymphoid tissue. DC were found to be essential for the maintenance of inducible bronchus-associated lymphoid tissue, a form of tertiary lymphoid tissue induced in the lungs of influenza virus-infected mice [22]. In this case, elimination of DC after the virus had been cleared from the lungs resulted in inducible bronchus-associated lymphoid tissue disintegration. Along this line, it was reported the formation of tumor-induced bronchus-associated lymphoid tissue in patients with non-small lung cancer, and that the density of mature DC in these structures is correlated with prolonged survival [23].

Other authors demonstrated that the intra-tumoral administration of genetically modified DC that expressed the chemokine CCL21, a chemoattractant for DC and naive T cells, generated effective T cell priming and measurable anti-tumor effects, independent of lymph node action [24]. A clinical study analyzing skin biopsies of patients inoculated intradermally with mature DC demonstrated that they induce changes to the microenvironment that enable the recruitment of naive and antigen-experienced central memory T cells [10]. In our system, the formation of the tertiary lymphoid structure depends on DC cocultured with Apo/Nec cells. It has been shown that *ex vivo* generation and Ag loading of DC allows to control the process of DC maturation, and to imprint in maturing DC functions essential for the preferential recruitment of distinct types of immune cells, thus inducing effective forms of cancer immunity [25–27]. DC-based cancer vaccines that have been tested successfully in murine models include DC loaded with different forms of tumor Ag [28,29]. Loading of DC can be achieved by different mechanisms, but more "natural" processing and epitope selection is expected from loading DC with a mixture of tumor Ags, as can be generated by DC coculture with apoptotic/necrotic tumor cells [30]. With Apo/Nec injections no important immune recruitment could be observed at the inoculation site. This lack of ability to attract other cells points out the relevance of a proper Ag presentation in order to mount a strong anti-tumor response.

Consequently, the repeated DC-Apo/Nec injections, which have previously phagocytosed and processed *ex vivo* tumor Ag, establish a state of chronic inflammation. This in turn induces the recruitment of PMN and macrophages to the vaccination site, which contribute to the inflammatory status. Then, a poor DC migration to the regional lymph nodes leave an "accumulation" of Ag-loaded DC at the injection site, that could be priming CD4 and CD8 T lymphocytes locally, contributing to establish a potent anti-tumoral immune response.

Acknowledgements

This work was supported by grants from the following institutions: CONICET, University of Buenos Aires, Agencia para el Desarrollo Científico y Tecnológico, Fundación Sales, Fundación para la Investigación y Prevención del Cáncer (FUCA) and Fundación María Calderón de la Barca, Argentina.

JM y RW are Career Investigators from CONICET; SMK is a fellow from CONICET.

References

[1] Melief CJ. Cancer immunotherapy by dendritic cells. Immunity 2008;29(September (3)):372–83.
[2] Palucka K, Ueno H, Fay J, Banchereau J. Harnessing dendritic cells to generate cancer vaccines. Ann N Y Acad Sci 2009;1174(September):88–98.
[3] Dillman RO, Selvan SR, Schiltz PM, McClay EF, Barth NM, DePriest C, et al. Phase II trial of dendritic cells loaded with antigens from self-renewing, proliferating autologous tumor cells as patient-specific antitumor vaccines in patients with metastatic melanoma: final report. Cancer Biother Radiopharm 2009;24(June (3)):311–9.
[4] von Euw EM, Barrio MM, Furman D, Levy EM, Bianchini M, Peguillet I, et al. A phase I clinical study of vaccination of melanoma patients with dendritic cells loaded with allogeneic apoptotic/necrotic melanoma cells. Analysis of toxicity and immune response to the vaccine and of IL-10-1082 promoter genotype as predictor of disease progression. J Transl Med 2008;6:6.
[5] Steinman RM. Dendritic cells in vivo: a key target for a new vaccine science. Immunity 2008;29(September (3)):319–24.
[6] Steinman RM. The dendritic cell system and its role in immunogenicity. Annu Rev Immunol 1991;9:271–96.
[7] Martin-Fontecha A, Lanzavecchia A, Sallusto F. Dendritic cell migration to peripheral lymph nodes. Handb Exp Pharmacol 2009;188:31–49.
[8] Austyn JM. New insights into the mobilization and phagocytic activity of dendritic cells. J Exp Med 1996;183(April (4)):1287–92.
[9] Caux C, Vanbervliet B, Massacrier C, Ait-Yahia S, Vaure C, Chemin K, et al. Regulation of dendritic cell recruitment by chemokines. Transplantation 2002;73(January (1 Suppl.)):S7–11.
[10] Schrama D, Pedersen LO, Keikavoussi P, Andersen MH, Straten PTP, Brocker EB, et al. Aggregation of antigen-specific T cells at the inoculation site of mature dendritic cells. J Invest Dermatol 2002;119(December (6)):1443–8.
[11] Goldszmid RS, Idoyaga J, Bravo AI, Steinman R, Mordoh J, Wainstok R. Dendritic cells charged with apoptotic tumor cells induce long-lived protective CD4+ and CD8+ T cell immunity against B16 melanoma. J Immunol 2003;171(December (11)):5940–7.
[12] Lappin MB, Weiss JM, Delattre V, Mai B, Dittmar H, Maier C, et al. Analysis of mouse dendritic cell migration in vivo upon subcutaneous and intravenous injection. Immunology 1999;98(October (2)):181–8.
[13] MartIn-Fontecha A, Sebastiani S, Hopken UE, Uguccioni M, Lipp M, Lanzavecchia A, et al. Regulation of dendritic cell migration to the draining lymph node: impact on T lymphocyte traffic and priming. J Exp Med 2003;198(August (4)):615–21.
[14] Eggert AA, Schreurs MW, Boerman OC, Oyen WJ, de Boer AJ, Punt CJ, et al. Biodistribution and vaccine efficiency of murine dendritic cells are dependent on the route of administration. Cancer Res 1999;59(July (14)):3340–5.
[15] Girard JP, Springer TA. High endothelial venules (HEVs): specialized endothelium for lymphocyte migration. Immunol Today 1995;16(September (9)):449–57.
[16] Schrama D, Voigt H, Eggert AO, Xiang R, Zhou H, Schumacher TN, et al. Immunological tumor destruction in a murine melanoma model by targeted LTalpha independent of secondary lymphoid tissue. Cancer Immunol Immunother 2008;57(January (1)):85–95.
[17] Irvine DJ, Stachowiak AN, Hori Y. Lymphoid tissue engineering: invoking lymphoid tissue neogenesis in immunotherapy and models of immunity. Semin Immunol 2008;20(April (2)):137–46.
[18] Aloisi F, Pujol-Borrell R. Lymphoid neogenesis in chronic inflammatory diseases. Nat Rev Immunol 2006;6(March (3)):205–17.
[19] Nasr IW, Reel M, Oberbarnscheidt MH, Mounzer RH, Baddoura FK, Ruddle NH, et al. Tertiary lymphoid tissues generate effector and memory T cells that lead to allograft rejection. Am J Transplant 2007;7(May (5)):1071–9.
[20] Humby F, Bombardieri M, Manzo A, Kelly S, Blades MC, Kirkham B, et al. Ectopic lymphoid structures support ongoing production of class-switched autoantibodies in rheumatoid synovium. PLoS Med 2009;6(January (1)):e1.
[21] Thaunat O, Nicoletti A. Lymphoid neogenesis in chronic rejection. Curr Opin Organ Transplant 2008;13(February (1)):16–9.
[22] GeurtsvanKessel CH, Willart MA, Bergen IM, van Rijt LS, Muskens F, Elewaut D, et al. Dendritic cells are crucial for maintenance of tertiary lymphoid structures in the lung of influenza virus-infected mice. J Exp Med 2009;206(October (11)):2339–49.
[23] Dieu-Nosjean MC, Antoine M, Danel C, Heudes D, Wislez M, Poulot V, et al. Long-term survival for patients with non-small-cell lung cancer with intratumoral lymphoid structures. J Clin Oncol 2008;26(September (27)):4410–7.
[24] Kirk CJ, Hartigan-O'Connor D, Mule JJ. The dynamics of the T-cell antitumor response: chemokine-secreting dendritic cells can prime tumor-reactive T cells extranodally. Cancer Res 2001;61(December (24)):8794–802.
[25] Kalinski P, Okada H. Polarized dendritic cells as cancer vaccines: directing effector-type T cells to tumors. Semin Immunol 2010;(April).
[26] Muthuswamy R, Urban J, Lee JJ, Reinhart TA, Bartlett D, Kalinski P. Ability of mature dendritic cells to interact with regulatory T cells is imprinted during maturation. Cancer Res 2008;68(July (14)):5972–8.
[27] Gustafsson K, Ingelsten M, Bergqvist L, Nystrom J, Andersson B, Karlsson-Parra A. Recruitment and activation of natural killer cells in vitro by a human dendritic cell vaccine. Cancer Res 2008;68(July (14)):5965–71.
[28] Lesterhuis WJ, Aarntzen EH, De Vries IJ, Schuurhuis DH, Figdor CG, Adema GJ, et al. Dendritic cell vaccines in melanoma: from promise to proof? Crit Rev Oncol Hematol 2008;66(May (2)):118–34.
[29] Banchereau J, Palucka AK. Dendritic cells as therapeutic vaccines against cancer. Nat Rev Immunol 2005;5(April (4)):296–306.
[30] Inzkirweli N, Guckel B, Sohn C, Wallwiener D, Bastert G, Lindner M. Antigen loading of dendritic cells with apoptotic tumor cell-preparations is superior to that using necrotic cells or tumor lysates. Anticancer Res 2007;27(July–August (4B)):2121–9.