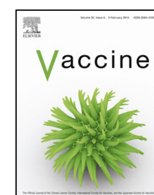
Vaccine 32 (2014) 1229–1232

Contents lists available at ScienceDirect

Vaccine

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



Brief report

CD207+ cells recruitment to the vaccination site and draining lymph nodes after the administration of DC-Apo/Nec vaccine in mice

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ARTICLE INFO

Article history:
Received 17 September 2013
Received in revised form 6 December 2013
Accepted 15 January 2014
Available online 29 January 2014

Keywords:
CD207+ cells
Dendritic cells
Vaccine
Ectopic lymphoid tissue
Melanoma

ABSTRACT

De novo ectopic lymphoid tissue formation is known to occur in certain disease and inflammatory settings. After an effective vaccination with dendritic cells (DC) charged with melanoma apoptotic/necrotic cells (Apo/Nec), a subcutaneous tertiary lymphoid structure was organized, where retained vaccine cells interacted with recruited inflammatory and T cells. In this work we report for the first time the recruitment of two morphologically different CD207+ cells to vaccination site. The time-course behavior of CD207+ cells was reciprocal between vaccination site and draining lymph nodes (DLNs). After 6–10 days, CD207+ cells localized at the paracortical region of DLNs, in close contact with T cell population. DLNs were enriched in a peculiar MHCII+ CD11c$^{(-)}$ CD207+ population, whose role remains to be determined. Whether CD207+ cells migration to the vaccination site can be associated with a differential anti-tumoral response remains as an open and exciting question.

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1. Introduction

Dendritic cells (DC) are the professional antigen-presenting cells of the immune system, with the potential to stimulate and regulate immune responses. Their special features regarding location, antigen handling, maturation and diversity have established their relevance in the development of vaccines [1]. Among the different available strategies, "loading" DC with tumor antigens and MHC-I and MHC-II-mediated antigen presentation to naïve T cells has been shown as an effective immunization approach [2]. We have previously shown that an experimental vaccine using DC charged with B16-F1 melanoma apoptotic/necrotic cells (Apo/Nec) was effective against tumor challenge [3]. After four doses of this vaccine (DC-Apo/Nec), a long-term systemic protection, dependent on CD4+ and CD8+ T cells, was attained against the development of melanoma tumors in mice [3,4]. Surprisingly, most of the cells were retained at the vaccination site, a dynamic structure that evolved to a tertiary lymphoid structure at five days post vaccination. A scarce migration of vaccine cells was detected to the draining lymph nodes (DLNs) after 24 h of the last vaccine dose [4]. Due to the recruitment of T lymphocytes in association with DC and the presence of Peripheral-Node-Addressin+ vessels, we hypothesized this structure to be functional and involved in the process of antitumoral protection. However, we have not addressed so far whether resident DC, such as skin DC, can participate in this process. In this work we assessed the recruitment and kinetics of host CD207+ (CD207 = Langerin) DC, such as Langerhans cells (LC) and related dermal DC, to lymph nodes and the vaccination site in DC-Apo/Nec-vaccinated mice.

2. Materials and methods

2.1. Animals

8–12 weeks-old C57BL/6 (HH-2b) mice were obtained from the animal facility at the Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires (Buenos Aires, Argentina). Mice were maintained in pathogen-free conditions, and studies were performed in accordance with local ethical guidelines.

2.2. Monoclonal antibodies, secondary staining reagents, and recombinant cytokines

Monoclonal antibodies against the following mouse antigens were used for immunostaining for flow cytometry and/or

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http://dx.doi.org/10.1016/j.vaccine.2014.01.025

immunohistochemistry (IHC) techniques: MHC-II (I-Ab) (AF6-120.1, mouse IgG2a BD Pharmigen™), CD207 (eBioL31, Rat IgG2a eBioscience, USA), PE-CD11c (HL3, Hamster IgG1, BD Pharmigen™). Isotype-matched controls used were: Rat IgG2a (R35-95, BD Pharmigen™), PE-Hamster IgG1 (BD Pharmigen™). Secondary staining reagents used included Biotin-anti-Rat Ig (BD Pharmigen™), Alexa 647-anti-Rat Ig (Goat anti-rat IgG H+L, Invitrogen™ Molecular Probes®), Cy5-anti-Rat IgG (H+L) (712-175-150, Donkey Ig, Jackson ImmunoResearch, USA), FITC-anti-Mouse Ig (Goat polyclonal F(ab')2, DakoCytomatin), streptavidin-horseradish peroxidase (Sav-HRP) (Anti-Rat Ig HRP Detection Kit, BD Pharmigen™). GM-CSF was obtained from PeproTech, Mexico DF, Mexico.

2.3. Culture of bone marrow-derived DC-Apo/Nec vaccine

DC-Apo/Nec vaccine was prepared as described in a previous report [4]. Briefly, bone marrow (BM) cells were harvested from femurs and tibias of healthy C57BL/6 mice; cultured 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. On day 7, DC were harvested by gentle pipetting, and co-cultured in DC conditioned medium with irradiated Apo/Nec B16 cells in a 1:1 ratio for 24 h. One dose per week of 2×10^5 DC-Apo/Nec was injected subcutaneously in the inguinal flank. The vaccination scheme always included 4 doses of vaccine.

2.4. DLNs single cell suspensions

To prepare single-cell suspensions from DLNs, inguinal lymph nodes were dissected and disaggregated mechanically and enzymatically with collagenase IV 0.82 mg/ml (Sigma-Aldrich, MO) and DNase I 1:10^6 (Invitrogen, Life Technologies). Single cell suspensions were obtained by filtration through a 100 μm-pore-size mesh.

2.5. Flow cytometry analysis

Samples were acquired with a BD FACS Aria II TM flow cytometer and data were analyzed with Cyflogic software (1.2.1 version, 1998).

2.6. Immunohistochemistry staining

Skin was obtained from the inguinal flank region, fixed in buffered formalin 10%, embedded in paraffin and sectioned 4–7 μm width for IHC. For CD207 staining, endogenous peroxidase was inactivated with 4% H_2O_2, and antigen unmasking was achieved by 20–30 min incubation at 95 °C in 10 mM citrate −0.05% Tween 20 buffer. Sections were blocked and stained according

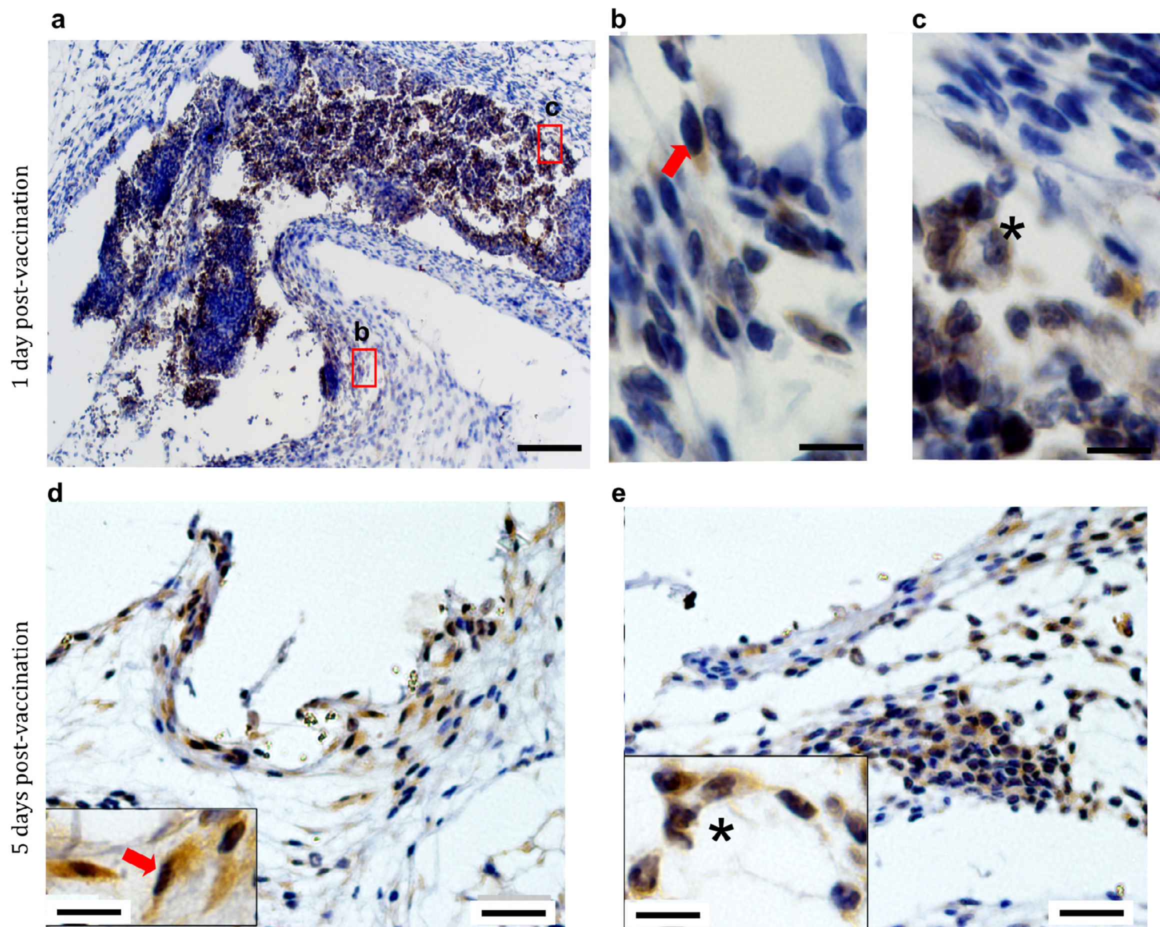

Fig. 1. CD207⁺ cells recruitment to the vaccination site. Detection by IHC of CD207⁺ cells at 1 day (a–c), or five days (d and e) after four doses of DC-Apo/Nec vaccine. (a) Upon vaccination, CD207⁺ cells observed at the surrounding inflammatory infiltrate were morphologically similar to fibroblasts, with fusiform nuclei (b, arrow), whereas those localized at the core of the vaccination site exhibited monocytic-like nuclei (c, asterisk). Five days later, there were signs of chronic inflammation (d and e), and the prevalence of both cell types clustered at the dermis, close to the tertiary structure (d and e). Scale bar represents: 100 μm (a); 20 μm (d and e), 10 μm (b and c, inset d and inset e).

to manufacturer instructions (Anti-Rat Ig HRP Detection Kit, BD PharmigenTM). Counterstaining was performed with hematoxylin.

3. Results and discussion

In order to evaluate CD207$^+$ cells response to vaccine administration, sections from the vaccination site were obtained at different time points after vaccination with four doses of DC-Apo/Nec, and immunostained for the presence of CD207$^+$ cells. As early as one day post-injection, the vaccination site showed signs of inflammation, swelling and tissue disruption. Polymorphonuclear leukocytes, necrotic, monocyte-like nucleated and apoptotic cells were observed in a highly dense cellular infiltrate, still not organized as a tertiary lymphoid structure (Fig. 1a). In this scenario, two morphologically different CD207$^+$ cells were found. Before the injection DC-Apo/Nec cells showed neither surface nor intracellular CD207 expression by flow cytometry (Fig. S1). CD207$^+$ cells with pyknotic, fusiform nuclei were detected at the surrounding inflammatory infiltrate (Fig. 1b, arrow), whereas CD207$^+$ cells with a monocytic-like nucleus (Fig. 1c, asterisk) were observed within the vaccine cell cumulus at the vaccination site. After five days, when tertiary lymphoid structure is present at the vaccination site, both types of cells were clustered in the surrounding dermis (Fig. 1d and e). The tissue showed signs of chronic inflammation, such as macrophage-histiocytes in hair bulbs (data not shown). The identity of these CD207$^+$ cell subsets needs to be further investigated, but as inflammation can involve DC populations that are not found in steady state, such recruitment could be due to monocyte-derived DCs attracted by the release of GM-CSF at these sites [5]. Preliminary results by our group showed that CD207$^+$ CD103$^+$ dermal DC would be enriched at the vaccination site compared to the non-vaccinated controls (unpublished results). Given the increasing relevance of this subset in skin-related immune responses [6,7], further description of DC subsets in our model remains an interesting and relevant aim.

The time-course behavior of CD207$^+$ cells was reciprocal between vaccination site and DLNs. The abundant infiltration in the dermis at early time points after vaccination (Fig. 1a) diminished after five days (Fig. 1 d and e) and was even lower at later time points (6–10 days) (data not shown). A hypothesis of their migration towards the DLNs in response to local stimulus was in agreement with the detection of an extensive infiltrate of CD207$^+$ cells, localized at the T-cell zone of the paracortex of vaccinated-mice DLNs (Fig. 2b and c). No differences were found in CD207$^+$ cells in the sinus of DLNs from both control and vaccinated mice (Fig. 2a and b). It has been shown that lymph node-resident DC are located preferentially in the cortical ridge, whereas migratory DC accumulate mostly in the deep zone of the paracortex [8,9]. In addition, the facts that mice from C57BL/6 strain lack the lymph-node resident CD207$^+$ DC population [10], and that DC-Apo/Nec vaccine cells do not express CD207 antigen (Fig. S1), suggest that the infiltrating cells here reported would be of migratory origin. In order to describe this population, we evaluated their phenotype by flow cytometry. DLNs from vaccinated mice were two-fold enriched in CD11c$^{(-)}$ CD207$^+$ cells, expressed as a percentage of MHC-II$^+$ cells (SD = 0.2, n = 2) (Fig. 2d). This population expressed higher levels of MHC-II antigen, as assessed by its Mean Fluorescence Intensity [MFI (DC-Apo/Nec)/control = 1.5, SD = 0.2]. The enriched population could be the result of CD11c down-regulation in a subset of DC [11]. In fact, this phenomenon was also observed in our

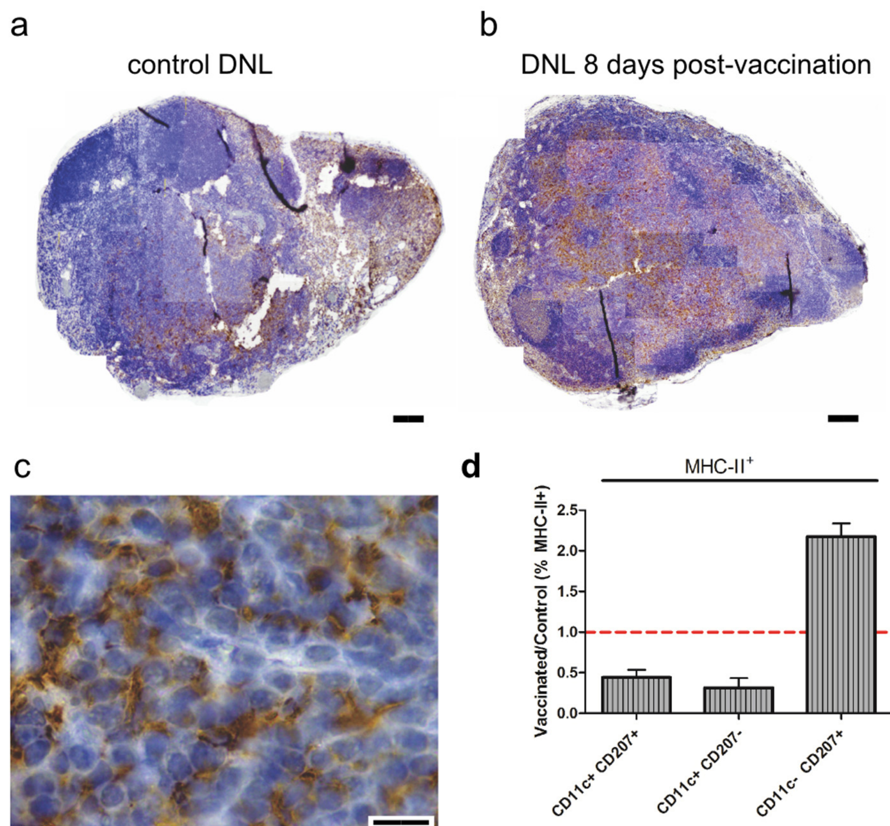

Fig. 2. CD207+ cells enrichment in DLNs from vaccinated mice. Detection by IHC of CD207$^+$ cells at 6–10 days after four doses of DC-Apo/Nec in DLNs from control (a) or vaccinated (b and c) mice. Images of DLNs (obtained as compositions of individual high resolution pictures taken at 400×) show an enrichment of CD207$^+$ cells in the T-cell paracortical area of DLNs of vaccinated mice (b). A strongly infiltrated T-cell area with CD207+ cells was observed in the deeper region of the paracortex (c), and was absent from most B-cell follicles. Scale bar represents 200 μm (a and b) and 10 μm (c). Flow cytometry analyses of DLNs. MHC-II$^+$ cells were analyzed for expression of CD11c and CD207 (d). Bars indicate the mean and SD of two independent experiments. A pool of DLNs from five mice was used for each condition.

BM-derived DC: CD11c expression was reduced from 89.3% to 59.3% after 24hs of co-culture with Apo/Nec cells (Fig. S1). Examples of immature migrating LC, that do not express typical mature-DC markers have been described in chronically inflamed skin in humans [12,13]. The migration of CD11c$^{(-)}$ cells to skin DLNs has also been reported in FITC-painting experiments, and their identity remains unsolved [14]. As DC-Apo/Nec vaccination elicits an efficient melanoma tumor rejection, in a context of scarce migration of vaccine cells to DLNs immediately after vaccination [4], these new results highlight the potential relevance of host CD207^{+} cells in the activation of DLNs.

4. Conclusions

Results reported here support the hypothesis that CD207^{+} cell accumulation in the vaccination site with posterior migration to DLNs, with DC-Apo/Nec cells failing to migrate efficiently, could be a host mechanism boosting the anti-tumoral response elicited by the DC-Apo/Nec vaccine. This work contributes to further characterize the components of the evolving tertiary lymphoid structure evoked in this immunotherapeutic vaccine model.

Conflict of interest statement

The authors state no conflict of interest. All authors have approved the final version of this article.

Acknowledgements

This work was supported by grants from the following institutions: University of Buenos Aires, Agencia Nacional de Promoción Científica y Tecnológica (PICT0010) and CONICET. We thank Dr. José Mordoh for carefully reading this manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2014.01.025.

References

[1] Steinman RM. Dendritic cells in vivo: a key target for a new vaccine science. Immunity 2008;29:319–24.

[2] Schuler-Thurner B. Rapid induction of tumor-specific type 1 T helper cells in metastatic melanoma patients by vaccination with mature, cryopreserved, peptide-loaded monocyte-derived dendritic cells. Journal of Experimental Medicine 2002;195:1279–88.

[3] 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. Journal of Immunology (Baltimore MD: 1950) 2003;171:5940–7.

[4] Mac Keon S, Gazzaniga S, Mallerman J, Bravo AI, Mordoh J, Wainstok R. Vaccination with dendritic cells charged with apoptotic/necrotic B16 melanoma induces the formation of subcutaneous lymphoid tissue. Vaccine 2010;28:8162–8.

[5] Ali OA, Emerich D, Dranoff G, Mooney DJ. In situ regulation of DC subsets and T cells mediates regression in mice. Science Translational Medicine 2009;1:1–22.

[6] Henri S, Poulin LF, Tamoutounour S, Ardouin L, Guilliams M, De Bovis B, et al. CD207+ CD103+ dermal dendritic cells cross-present keratinocyte-derived antigens irrespective of the presence of Langerhans cells. Journal of Experimental Medicine 2010;207:189–206.

[7] Stoecklinger A, Eticha TD, Mesdaghi M, Kissenpfennig A, Malissen B, Thalhamer J, et al. Langerin+ dermal dendritic cells are critical for CD8 T cell activation and IgH γ-1 class switching in response to gene gun vaccines. Journal of Immunology (Baltimore, MD: 1950) 2011;186:1377–83.

[8] Kissenpfennig A, Henri S, Dubois B, Laplace-Builhé C, Perrin P, Romani N, et al. Dynamics and function of Langerhans cells in vivo: dermal dendritic cells colonize lymph node areas distinct from slower migrating Langerhans cells. Immunity 2005;22:643–54.

[9] Sixt M, Kanazawa N, Selg M, Samson T, Roos G, Reinhardt DP, et al. The conduit system transports soluble antigens from the afferent lymph to resident dendritic cells in the T cell area of the lymph node. Immunity 2005;22:19–29.

[10] Flacher V, Tripp CH, Stoitzner P, Haid B, Ebner S, Del Frari B, et al. Epidermal Langerhans cells rapidly capture and present antigens from C-type lectin-targeting antibodies deposited in the dermis. Journal of Investigative Dermatology 2010;130:755–62.

[11] Singh-Jasuja H, Thiolat A, Ribon M, Boissier M-C, Bessis N, Rammensee H-G, et al. The mouse dendritic cell marker CD11c is down-regulated upon cell activation through Toll-like receptor triggering. Immunobiology 2013;218:28–39.

[12] Geissmann F, Dieu-Nosjean MC, Dezutter C, Valladeau J, Kayal S, Leborgne M, et al. Accumulation of immature Langerhans cells in human lymph nodes draining chronically inflamed skin. Journal of Experimental Medicine 2002;196:417–30.

[13] Chalermsarp N, Azuma M. Identification of three distinct subsets of migrating dendritic cells from oral mucosa within the regional lymph nodes. Immunology 2009;127:558–66.

[14] Douillard P, Stoitzner P, Tripp CH, Clair-Moninot V, Aït-Yahia S, McLellan AD, et al. Mouse lymphoid tissue contains distinct subsets of langerin/CD207 dendritic cells, only one of which represents epidermal-derived Langerhans cells. Journal of Investigative Dermatology 2005;125:983–94.