Published OnlineFirst February 9, 2010; DOI: 10.1158/0008-5472.CAN-09-3276

Review

Cancer Research

Dendritic Cell-Derived Exosomes for Cancer Immunotherapy: What's Next?

Sophie Viaud[1,2], Clotilde Théry[3,4,5], Stéphanie Ploix[2,5], Thomas Tursz[1,2], Valérie Lapierre[2,6], Olivier Lantz[3,4,5], Laurence Zitvogel[1,2,5,7], and Nathalie Chaput[1,2,5,6]

Abstract

Exosomes are nanovesicles originating from late endosomal compartments and secreted by most living cells in *ex vivo* cell culture conditions. The interest in exosomes was rekindled when B-cell and dendritic cell-derived exosomes were shown to mediate MHC-dependent immune responses. Despite limited understanding of exosome biogenesis and physiological relevance, accumulating evidence points to their bioactivity culminating in clinical applications in cancer. This review focuses on the preclinical studies exploiting the immunogenicity of dendritic cell-derived exosomes (Dex) and will elaborate on the past and future vaccination trials conducted using Dex strategy in melanoma and non-small cell lung cancer patients. *Cancer Res; 70(4); 1281–5. ©2010 AACR.*

Introduction

Exosomes were first described as vesicles secreted by reticulocytes allowing the elimination of obsolete molecules such as transferrin receptors (1, 2). Intense research ensued when B-cell-derived exosomes were shown to stimulate MHC class II-dependent $CD4^+$ T cells *in vitro* (3, 4). Since then, many studies highlighted that dendritic cell (DC)-derived exosomes (Dex) could modulate immune responses (4, 5), either directly by exposing MHC and costimulatory molecules or indirectly by conveying internal components to surrounding cells (6). Importantly, exosomes seem to also transfer nucleic acids such as mRNA and microRNA and may represent a new mechanism of genetic exchange between cells (4, 7). Despite significant advances achieved in delineating their protein (8) and lipid composition (9) and their biogenesis (10), their physiopathological relevance remains unclear. Two studies on placental exosomes indicated that such vesicles exhibiting T-cell inhibitory potential through various mechanisms (such as the role of FasL; ref. 11; and ULBP molecules; ref. 12) may be associated with term delivery during pregnancy (11). An elegant work pointed out that delivering antigens *in vivo* through small secreted vesicles such as exosomes is more immunogenic than the mere delivery of its soluble form in tumor models (13). The increasing knowledge about the biological effects of exosomes provides exciting prospects for exosome development in therapy. Here, we will focus on Dex and their capacity to induce immune responses in tumor-bearing hosts, on the possibility of enhancing Dex bioactivity ("second generation" Dex), and finally we will introduce our future phase II clinical trial testing the clinical efficacy of second generation Dex.

Dex as Cell-free Vaccines for Cancer Treatment

In 1998, we showed in mouse models that Dex pulsed with peptide acid eluted from a variety of different tumors mediated tumor growth retardation in an MHC- and $CD8^+$ T-cell-dependent manner (5). Since then, the mechanisms implicated in the bioactivity of Dex *in vitro* and *in vivo* were further dissected. Indeed, most lines of experimental evidence converge toward the demonstration of the indirect capacity of exosomal MHC class I and II molecules to trigger CD8+ and CD4+ T-cell activation. Dex can transfer functional peptide-loaded MHC class I and II complexes to DCs *in vitro* (6, 14). *In vivo* studies of exosome transfer indicated that Dex require *ex vivo* generated (14) or host DC (15). We next analyzed the molecular mechanisms involved in the transfer of exosomal MHC molecules to host APC and the role played by the maturation status of DC for the Dex-mediated immunogenicity *in vivo*. The analysis of the Dex protein composition revealed that Dex are enriched in a variety of proteins that could potentially dock their membrane to that of host APC. Indeed, exosomal milk fat globule-EGF factor VIII/MFG-E8 (theoretically binding to the integrins $\alpha v\beta 5$ and $\alpha v\beta 3$; refs. 8, 16, 17), as well as Mac-1 α-chain (CD11b) and tetraspanins (CD9, CD63, CD82, CD81), were tested for their potential role in the immunogenicity of Dex (at least when mouse bone marrow-derived

Authors' Affiliations: [1]Institut National de la Santé et de la Recherche Médicale U805, Villejuif, France; [2]Institut Gustave Roussy, Villejuif, France; [3]Institut Curie; [4]Institut National de la Santé et de la Recherche Médicale, U932 Paris, France; [5]Centre d'Investigation Clinique en Biothérapie, CICBT 507; [6]Laboratoire de thérapie cellulaire, Institut Gustave Roussy, Villejuif, France; and [7]Faculté de Médecine de l'université Paris-Sud XI, Le Kremlin-Bicêtre, France

Corresponding Author: Nathalie Chaput or Laurence Zitvogel, Centre d'Investigation Clinique Biothérapie 507, Laboratoire de Thérapie Cellulaire, Institut Gustave Roussy, 39 rue Camille Desmoulins, 94805 Villejuif, France. Phone: 33-1-42-11-66-16; Fax: 33-1-42-11-60-94; E-mail: nathalie.chaput@igr.fr or laurence.zitvogel@igr.fr.

doi: 10.1158/0008-5472.CAN-09-3276

Published OnlineFirst February 9, 2010; DOI: 10.1158/0008-5472.CAN-09-3276

DC were used as recipients; ref. 18).[8] These proteins did not seem to be involved (19), whereas the interaction between Dex and CD8α$^+$ DC was shown to be mediated through ICAM-1/LFA-1 molecules (20). Dex transfer onto DC did not mediate DC maturation *in vitro* (6).[9] These data were indirectly supported by two lines of evidence pointing out that Dex secreted from immature DC (imDex) failed to induce potent T-cell responses.

First, Dex produced by imDex express low level of molecules implicated in direct T-cell activation or DC targeting such as CD40, CD80, CD86, and ICAM-1, respectively (19–23). Secondly, imDex were poorly immunogenic in cancer-bearing mice and patients. In HHD2 mice, Dex loaded with Mart-1 peptides required TLR9L adjuvants to mount Mart-1-specific CD8$^+$ T-cell activation leading to tumor regression (15). In the first clinical trials using imDex as cell-free vaccines in advanced melanoma (24) and lung cancer-bearing patients (25), we failed to detect vaccine-specific T-cell responses while observing potent Dex-related NK cell activation.

Hence, Dex not only mediate bioactivities on T lymphocytes but also modulate in the innate arm of immune responses. Although tumor-derived exosomes (Tex) could inhibit NK cells through blockade of IL-2-mediated NK cell activation leading to tumor escape (26) or through down regulation of natural killer group 2 member D (NKG2D) on peripheral blood lymphocytes leading to decreased T-cell cytotoxicity (27, 28), Dex can directly trigger NK cell activation in mice and cancer patients. Indeed, we showed that Dex secreted from bone marrow-derived or monocyte-derived DC harbor functional membrane bound NKG2D ligands and interleukin (IL)-15Rα. Importantly, the first phase I clinical study revealed that Dex vaccines significantly augmented circulating NK cell numbers and NKG2D-dependent functions in the majority of melanoma patients (29).

Modulation of Dex Immunogenicity: Second Generation Dex

Over the past 10 years, many groups have been interested in finding a way of using and improving exosomes as immunological tools (Fig. 1A). Because DC represent a promising cell-based strategy to elicit or enhance antitumor immune responses, investigators started to think about the pros and cons of Dex versus DC therapy. Dex are unarguably stable vesicles harboring defined protein and lipid contents that could be tailor-manufactured from genetically modified and GMP-certified cell lines. Furthermore, on the basis of the two first feasibility trials, we generated about 10 vaccines of NK cell-stimulating Dex from one leukapheresis in most individuals, suggesting that Dex may at least complement DC-based therapies of cancer and represent a valuable strategy to boost DC-mediated T- and NK-cell responses. Therefore, the question arose of how to influence Dex bioactivity.

Although the issues of dose and route were studied by some investigators (15, 24, 25, 30), such considerations may be best addressed in future trials aimed at monitoring Dex bioactivity in humans.

In contrast, many studies aimed at changing the molecular composition of exosomes were conducted in order to influence their immune properties. Thus, between 2005 and 2007, Robbins and colleagues attempted to generate tolerogeneic Dex using immature DC treated or transduced with adenoviral vector encoding IL-10, IL-4, or FasL, which were able to suppress inflammation in a murine model of delayed-type hypersensibility and reduce the severity of established collagen-induced arthritis (31–35). Likewise, Dex bearing donor-MHC antigens delayed chronic rejection when transferred into a rat model of cardiac allograft (36), and when produced by TGF-β1- and IL-10-treated DC, Dex were efficient inducers of immune tolerance in a murine skin transplantation model (37). More recently, Dex secreted from DC overexpressing indoleamine 2,3-dioxygenase (IDO) reduced inflammation in a model of rheumatoid arthritis (32).

Likewise, Dex can be directly or indirectly (via DC) loaded with desired immunogens to redirect and gear the immune response. Aline and colleagues showed that mice immunized with Dex loaded with *Toxoplasma gondii* antigen were protected against *T. gondii* chronic infection (38). Humoral responses directed against diphtheria toxin were induced by DT-pulsed Dex in naïve mice (39). Dex produced by DC pulsed with acid-eluted tumor peptides reduced tumor growth in mice (5). The question of whether Dex is more potent at raising CD4+ and/or CD8+ T-cell responses when antigens are directly versus indirectly loaded onto exosomes has been addressed by us and others. In an MHC class II-dependent TCR transgenic T-cell model, Qazi and colleagues showed that B- and T-cell responses can only be triggered *in vivo* when Dex are indirectly loaded through DC pulsing and concluded that in such conditions, Dex exert potent antigen-specific Th1 responses *in vivo* (40). When comparing the MHC class II-dependent immunogenicity of Dex secreted from immature versus mature DC in skin transplantation models, we established the superiority of LPS-matured DC-derived exosomes, which was accounted for by their enrichment in ICAM-1 and CD86 molecules (20). Our group has implemented these results with human cells and generated Dex from mature DC cultures. Such mDex offer advantages compared with imDex. First, mDex exhibit increased expression of MHC II molecules, CD40, ICAM-1, IL-15Rα, and NKG2D ligands. Second, mDex were indirectly loaded with MHC class I and II peptide antigens through maturing DC leading to potent T-cell triggering in the absence of recipient DC.[10]

[8] Théry C. Unpublished data.
[9] Zitvogel L. Unpublished data.
[10] Chaput N. Unpublished data.

Published OnlineFirst February 9, 2010; DOI: 10.1158/0008-5472.CAN-09-3276

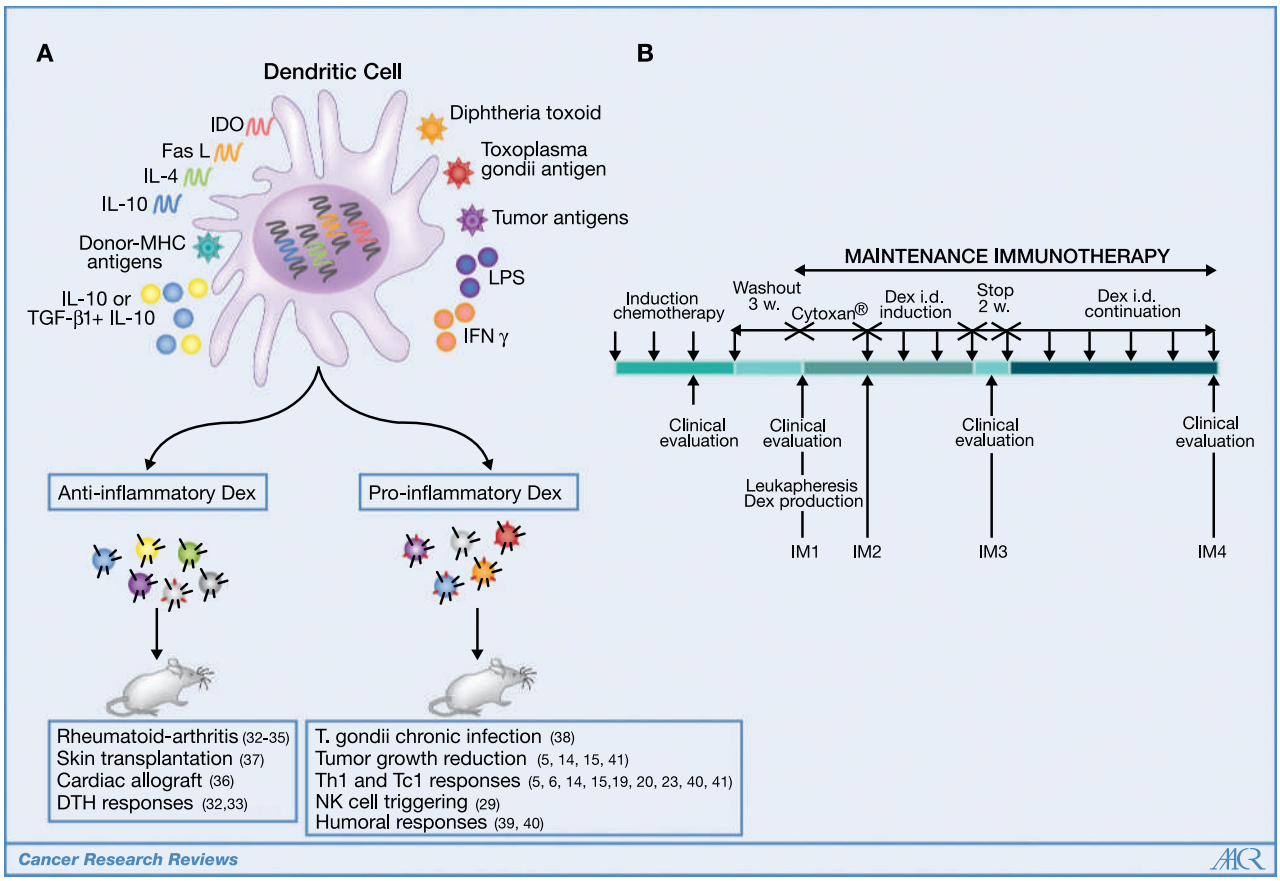

Figure 1. Bioactivity of exosomes as a function of the maturation state of the secretory dendritic cell. A, different strategies for the production of Dex with pro- or anti-inflammatory properties. B, schematic of the future maintenance immunotherapy clinical trial testing the clinical efficacy of second generation Dex in patients bearing NSCLC. IM, immunomonitoring; w., week, i.d., intradermally. The references to the corresponding Dex-applications (pro- or anti-inflammatory) are shown in brackets.

Clinical Trial Using Second Generation Dex in Non–Small Cell Lung Cancer Patients

A phase II clinical trial testing the clinical benefit of Dex as maintenance immunotherapy in patients bearing inoperable (stage IIIB to IV) non small cell lung cancer (NSCLC) responded to or stabilized after induction chemotherapy was launched in November 2009 at the Gustave Roussy and Curie Institutes (Fig. 1B). Dex will be purified from autologous maturing MD-DC loaded with HLA-DP*04-restricted (MAGE-3) and HLA-A*02-restricted peptides (NY-ESO-1, MAGE-1, MAGE-3, MART-1). Patients will first receive four cycles of platinum-based chemotherapy. Based on our preclinical (41) and clinical data (42) showing that metronomic cyclophosphamide (CTX) facilitates Dex-mediated T-cell priming and restores T- and NK-cell functions in end stage patients, HLA-A*02 responders will be eligible for the induction immunotherapy on the basis of the combination of a 3-week oral therapy with low dose CTX (42) followed by four weekly intradermal Dex injections. Continuation immunotherapy will maintain Dex vaccines every 2 weeks for 6 weeks. Forty-one patients will be enrolled between November 2009 and October 2011. The primary objective is to ameliorate the rate of progression-free survival at 4 months postchemotherapy. Secondary objectives are the clinical efficacy of Dex (assessed as overall survival, objective response rates), the biomarkers of efficacy (NK activation, restoration of NKG2D expression, and peptide vaccine-specific T-cell responses), and the safety of mDex in this cohort.

Conclusion

Dex are very promising vaccines in various physiopathological contexts. It is now possible to manipulate the cells from which Dex originate to modulate Dex bioactivity. Dex are able to activate adaptive (6, 15, 40, 41) and innate immunity (29). In addition, preclinical studies showed that peptide vaccines have a higher antitumor efficacy when carried by exosomes (15, 41). However, Dex are vesicles derived from DCs and therefore, their production requires a clinical cell therapy unit qualified for production according to good manufacturing practices with skilled and experienced employees. Moreover, DC differentiation involves growth factors and maturation agents that make the process expensive. However,

Published OnlineFirst February 9, 2010; DOI: 10.1158/0008-5472.CAN-09-3276

Dex are produced in large quantities allowing several vaccines. Finally, Dex are very stable and can be *cryo*-preserved more than 6 months at −80°C with a phenotype and function preserved. This stability gives them an advantage over DCs. Research and development of Dex is timely because phase III trials show the clinical benefit of peptides and antigen-loaded DC in melanoma and prostate cancer, respectively. Dex could substitute or boost other strategies of immunotherapy or be used as a maintenance vaccine. However, future prospects should aim at designing and engineering synthetic exosomes for broader therapeutic indications as has been recently proposed (43).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Grant Support

Institut National de la Santé et de la Recherche Médicale (INSERM), Institut Gustave Roussy, Institut Curie, l'Institut National du Cancer, la Fondation Bettencourt Schueller, and donations (Elisabeth Badinter, Agnès b.), Fondation de France.

Received 9/8/09; revised 11/2/09; accepted 11/4/09; published OnlineFirst 2/9/10.

References

1. Harding C, Heuser J, Stahl P. Receptor-mediated endocytosis of transferrin and recycling of the transferrin receptor in rat reticulocytes. J Cell Biol 1983;97:329–39.
2. Pan BT, Teng K, Wu C, Adam M, Johnstone RM. Electron microscopic evidence for externalization of the transferrin receptor in vesicular form in sheep reticulocytes. J Cell Biol 1985;101:942–8.
3. Raposo G, Nijman HW, Stoorvogel W, et al. B lymphocytes secrete antigen-presenting vesicles. J Exp Med 1996;183:1161–72.
4. Thery C, Ostrowski M, Segura E. Membrane vesicles as conveyors of immune responses. Nat Rev Immunol 2009;9:581–93.
5. Zitvogel L, Regnault A, Lozier A, et al. Eradication of established murine tumors using a novel cell-free vaccine: dendritic cell-derived exosomes. Nat Med 1998;4:594–600.
6. Thery C, Duban L, Segura E, Veron P, Lantz O, Amigorena S. Indirect activation of naive CD4+ T cells by dendritic cell-derived exosomes. Nat Immunol 2002;3:1156–62.
7. Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ, Lotvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol 2007;9:654–9.
8. Thery C, Regnault A, Garin J, et al. Molecular characterization of dendritic cell-derived exosomes. Selective accumulation of the heat shock protein hsc73. J Cell Biol 1999;147:599–610.
9. Laulagnier K, Motta C, Hamdi S, et al. Mast cell- and dendritic cell-derived exosomes display a specific lipid composition and an unusual membrane organization. Biochem J 2004;380:161–71.
10. Buschow SI, Nolte-'t Hoen EN, van Niel G, et al. MHC II in dendritic cells is targeted to lysosomes or T cell-induced exosomes via distinct multivesicular body pathways. Traffic 2009;10:1528–42.
11. Taylor DD, Akyol S, Gercel-Taylor C. Pregnancy-associated exosomes and their modulation of T cell signaling. J Immunol 2006;176:1534–42.
12. Hedlund M, Stenqvist AC, Nagaeva O, et al. Human placenta expresses and secretes NKG2D ligands via exosomes that down-modulate the cognate receptor expression: evidence for immunosuppressive function. J Immunol 2009;183:340–51.
13. Zeelenberg IS, Ostrowski M, Krumeich S, et al. Targeting tumor antigens to secreted membrane vesicles *in vivo* induces efficient antitumor immune responses. Cancer Res 2008;68:1228–35.
14. Andre F, Chaput N, Schartz NE, et al. Exosomes as potent cell-free peptide-based vaccine. I. Dendritic cell-derived exosomes transfer functional MHC class I/peptide complexes to dendritic cells. J Immunol 2004;172:2126–36.
15. Chaput N, Schartz NE, Andre F, et al. Exosomes as potent cell-free peptide-based vaccine. II. Exosomes in CpG adjuvants efficiently prime naive Tc1 lymphocytes leading to tumor rejection. J Immunol 2004;172:2137–46.
16. Andersen MH, Berglund L, Rasmussen JT, Petersen TE. Bovine PAS-6/7 binds alpha v beta 5 integrins and anionic phospholipids through two domains. Biochemistry 1997;36:5441–6.
17. Taylor MR, Couto JR, Scallan CD, Ceriani RL, Peterson JA. Lactadherin (formerly BA46), a membrane-associated glycoprotein expressed in human milk and breast carcinomas, promotes Arg-Gly-Asp (RGD)-dependent cell adhesion. DNA Cell Biol 1997;16:861–9.
18. Veron P, Segura E, Sugano G, Amigorena S, Thery C. Accumulation of MFG-E8/lactadherin on exosomes from immature dendritic cells. Blood Cells Mol Dis 2005;35:81–8.
19. Segura E, Amigorena S, Thery C. Mature dendritic cells secrete exosomes with strong ability to induce antigen-specific effector immune responses. Blood Cells Mol Dis 2005;35:89–93.
20. Segura E, Nicco C, Lombard B, et al. ICAM-1 on exosomes from mature dendritic cells is critical for efficient naive T-cell priming. Blood 2005;106:216–23.
21. Clayton A, Court J, Navabi H, et al. Analysis of antigen presenting cell derived exosomes, based on immuno-magnetic isolation and flow cytometry. J Immunol Methods 2001;247:163–74.
22. Segura E, Guerin C, Hogg N, Amigorena S, Thery C. CD8+ dendritic cells use LFA-1 to capture MHC-peptide complexes from exosomes *in vivo*. J Immunol 2007;179:1489–96.
23. Sprent J. Direct stimulation of naive T cells by antigen-presenting cell vesicles. Blood Cells Mol Dis 2005;35:17–20.
24. Escudier B, Dorval T, Chaput N, et al. Vaccination of metastatic melanoma patients with autologous dendritic cell (DC) derived-exosomes: results of thefirst phase I clinical trial. J Transl Med 2005;3:10.
25. Morse MA, Garst J, Osada T, et al. A phase I study of dexosome immunotherapy in patients with advanced non-small cell lung cancer. J Transl Med 2005;3:9.
26. Liu C, Yu S, Zinn K, et al. Murine mammary carcinoma exosomes promote tumor growth by suppression of NK cell function. J Immunol 2006;176:1375–85.
27. Clayton A, Mitchell JP, Court J, Linnane S, Mason MD, Tabi Z. Human tumor-derived exosomes down-modulate NKG2D expression. J Immunol 2008;180:7249–58.
28. Clayton A, Tabi Z. Exosomes and the MICA-NKG2D system in cancer. Blood Cells Mol Dis 2005;34:206–13.
29. Viaud S, Terme M, Flament C, et al. Dendritic cell-derived exosomes promote natural killer cell activation and proliferation: a role for NKG2D ligands and IL-15Ralpha. PLoS One 2009;4:e4942.
30. Hao S, Ye Z, Yang J, Bai O, Xiang J. Intradermal vaccination of dendritic cell-derived exosomes is superior to a subcutaneous one in the induction of antitumor immunity. Cancer Biother Radiopharm 2006;21:146–54.
31. Bianco NR, Kim SH, Morelli AE, Robbins PD. Modulation of the immune response using dendritic cell-derived exosomes. Methods Mol Biol 2007;380:443–55.
32. Bianco NR, Kim SH, Ruffner MA, Robbins PD. Therapeutic effect of exosomes from indoleamine 2,3-dioxygenase-positive dendritic cells in collagen-induced arthritis and delayed-type hypersensitivity disease models. Arthritis Rheum 2009;60:380–9.
33. Kim SH, Bianco N, Menon R, et al. Exosomes derived from genetically modified DC expressing FasL are anti-inflammatory and immunosuppressive. Mol Ther 2006;13:289–300.

Published OnlineFirst February 9, 2010; DOI: 10.1158/0008-5472.CAN-09-3276

34. Kim SH, Bianco NR, Shufesky WJ, Morelli AE, Robbins PD. Effective treatment of inflammatory disease models with exosomes derived from dendritic cells genetically modified to express IL-4. J Immunol 2007;179:2242–9.
35. Kim SH, Lechman ER, Bianco N, et al. Exosomes derived from IL-10-treated dendritic cells can suppress inflammation and collagen-induced arthritis. J Immunol 2005;174:6440–8.
36. Peche H, Renaudin K, Beriou G, Merieau E, Amigorena S, Cuturi MC. Induction of tolerance by exosomes and short-term immunosuppression in a fully MHC-mismatched rat cardiac allograft model. Am J Transplant 2006;6:1541–50.
37. Liu YY, Fan HH, Ren YN, et al. [Immune tolerance induced by exosomes derived from regulatory dendritic cells of mice]. Zhongguo Shi Yan Xue Ye Xue Za Zhi 2008;16:406–10.
38. Aline F, Bout D, Amigorena S, Roingeard P, Dimier-Poisson I. Toxoplasma gondii antigen-pulsed-dendritic cell-derived exosomes induce a protective immune response against T. gondii infection. Infect Immun 2004;72:4127–37.
39. Colino J, Snapper CM. Exosomes from bone marrow dendritic cells pulsed with diphtheria toxoid preferentially induce type 1 antigen-specific IgG responses in naive recipients in the absence of free antigen. J Immunol 2006;177:3757–62.
40. Qazi KR, Gehrmann U, Domange Jordo E, Karlsson MC, Gabrielsson S. Antigen-loaded exosomes alone induce Th1-type memory through a B-cell-dependent mechanism. Blood 2009;113:2673–83.
41. Taieb J, Chaput N, Schartz N, et al. Chemoimmunotherapy of tumors: cyclophosphamide synergizes with exosome based vaccines. J Immunol 2006;176:2722–9.
42. Ghiringhelli F, Menard C, Puig PE, et al. Metronomic cyclophosphamide regimen selectively depletes CD4+CD25+ regulatory T cells and restores T and NK effector functions in end stage cancer patients. Cancer Immunol Immunother 2007;56:641–8.
43. De La Pena H, Madrigal JA, Rusakiewicz S, et al. Artificial exosomes as tools for basic and clinical immunology. J Immunol Methods 2009;344:121–32.

Cancer Research
The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

AACR American Association for Cancer Research

Dendritic Cell-Derived Exosomes for Cancer Immunotherapy: What's Next?

Sophie Viaud, Clotilde Théry, Stéphanie Ploix, et al.

Cancer Res 2010;70:1281-1285. Published OnlineFirst February 9, 2010.