Atlas of Genetics and Cytogenetics in Oncology and Haematology

OPEN ACCESS JOURNAL AT INIST-CNRS

Deep Insight Section

Natural nanoparticules against cancer: mature dendritic cell-derived exosomes

Sophie Viaud, Mélanie Desbois, Stéphanie Ploix, Laurence Zitvogel, Nathalie Chaput

Institut Gustave Roussy, Villejuif, France (SV, MD, SP, LZ, NC); Institut National de la Sante et de la Recherche Medicale, U1015, Villejuif, France (SV, LZ); Centre d'Investigation Clinique en Biotherapie des cancers, CIC BT 507, Villejuif, France (MD, SP, LZ, NC); Universite de medecine du Kremlin Bicetre, Paris XI, Kremlin Bicetre, France (MD, LZ); Laboratoire de Therapie Cellulaire, Villejuif, France (NC)

Published in Atlas Database: May 2010

Online updated version : http://AtlasGeneticsOncology.org/Deep/DexandCancerID20078.html
DOI: 10.4267/2042/44973

Abstract

Dendritic cell-derived exosomes (Dex) are nanoparticules (50-100 nm in diameter) originating from late endosomes. These vesicles are secreted by living dendritic cells (DCs) in the extracellular milieu and have demonstrated the capacity to activate innate and adaptive immunity. These immuno-stimulatory properties have stimulated researchers to use Dex as a cell free vaccine. The development of a production process following good laboratory practices has allowed the use of Dex in vaccination protocols in patients in the early 2000s. However, no modulation of adaptive immune responses was observed in these clinical trials. Thus the scientific community attempted to decipher mechanisms of action involved in the establishment of an effective immune response after Dex vaccination. The results stressed that the immunogenicity of Dex reflects the state of maturation of DCs. Taking into account these new knowledge, our team developed a new generation of Dex capable of inducing immune cellular responses against tumor antigens. Their effectiveness is currently being evaluated in patients suffering from non small cell lung cancer.

Running title: Dendritic cell-derived exosomes as cell free vaccine
Key words: Dendritic cell, exosomes, cancer, immunotherapy
Abbreviations: Dendritic cells (DC), DC-derived exosomes (Dex), Natural killer (NK), Toll like receptor (TLR), Antigen presenting cell (APC), Monocyte derived-dendritic cells (MD-DC).

I) Dendritic cell-derived exosomes: from basic science to clinical trial

The interest for Dex began in 1998 when it was shown that these vesicles secreted into the culture supernatant of immature dendritic cells (immature Dex or *"iDex"*) could induce stabilization and regression of tumors in mice. These antitumor effects induced by *iDex* pulsed with tumor antigens were dependant on $CD8^+$ T cells in mice (Zitvogel et al., 1998). Subsequently, it was demonstrated that *iDex* could activate $CD4^+$ T cells by transferring MHC-II/antigen complexes to a recipient DC (Théry et al., 2002) and that human *iDex* could also transfer MHC-I/antigen complexes to a recipient DC for the polarization of Tc1 $CD8^+$ T cells (André et al., 2004). Thus, *iDex* do not activate T lymphocytes directly but need to be transferred on DCs. Studying different population of DCs that could be able to present Dex for the initiation of $CD4^+$ T cell responses, $CD8\alpha^-$ DCs were demonstrated to have the ability to present *iDex's* MHC-II/antigen complexes *in vitro* (Théry et al., 2002). However, *in vivo* data suggested that endogenous $CD8\alpha^+$ DCs were the main recipients of exosomes without the need for internalization and processing, LFA-1 being required for Dex capture (Segura et al., 2007). Moreover, the necessity to administer an endogenous DCs' maturation agent (ie TLR-3 or -9) along with *iDex*, *in vivo* in mice, confirmed that Dex's MHC/antigen complexes required the intervention of an antigen-presenting cell to initiate a Tc1 immune response (Chaput et al., 2004). Therefore, *iDex* promote the exchange of functional MHC/antigen complexes between DCs. Such a mechanism could increase the number of antigen

presenting DCs thus amplifying the initiation of adaptive immune responses. These results provide a rational for the use of *iDex* as a therapeutic vaccine in patients bearing advanced cancer.

After the development of a production method following good laboratory practices (Hsu et al., 2003; Lamparski et al., 2002), two phase I clinical trials using autologous *iDex* pulsed with MAGE-3 peptides in patients with stage III/IV melanoma (French trial) and lung cancer (US trial) allowed us to monitor immunological responses. At this time *iDex* were injected without any adjuvant. These trials aimed at evaluating the feasibility of Dex production by autologous monocyte-derived DCs and the safety of Dex inoculation. Secondary endpoints were the immunomonitoring of peptide specific $CD4^+$ and $CD8^+$ T cell responses restricted by exosomal MHC class II and I molecules. Fifteen patients were included in the french trial and thirteen in the US trial. Feasibility and tolerance were good; however, no T cell responses were pointed out after *iDex* vaccinations (Escudier et al., 2005; Morse et al., 2005). The discrepancy between some clinical responses and the lack of any detectable T cell responses (Tetramer stainings, Elispots) prompted the search for alternate effector mechanisms accounting for the tumoricidal activity. We studied NK activity in patients enrolled in these clinical trials and we could show that *iDex* were able to restore NK cell activity in half of treated patients (Chaput et al., 2006; Viaud et al., 2009). All of these preclinical and clinical studies have led to improve this vaccine approach in order to obtain Dex that could activate an effective adaptive immune response *in vivo*.

II) Strategies to improve Dex immunogenicity

Three strategies were explored to improve the antitumor efficacy of Dex: as already mentioned above the concomitant injection of an immunological adjuvant (TLR-3 or -9) (Chaput et al., 2004; Guo et al., 2008), inhibition of $CD4^+CD25^+Foxp3^+$ regulatory T cells (Treg) (Taïeb et al., 2006) and finally the use of mature DC to produce Dex (mDEX) (Bianco et al., 2007; Qazi et

al., 2009; Segura et al., 2005a, Segura et al., 2005b; Sprent, 2005). The role of Treg in restricting T cell based-immune responses has gained renewed recognition. Cyclophosphamide (CTX) at immuno-potentiating doses has been shown to inhibit $CD4^+CD25^+$ Treg cell activity (Ghiringhelli et al., 2004; Ghiringhelli et al., 2007). Therefore, we investigated the combined effects of CTX and *iDex* to elicit antitumor immunity leading to tumor rejection in the poorly immunogenic B16F10 melanoma model in mice (Taïeb et al., 2006). *iDex*-mediated anti-tumor effects were dramatically improved in CTX treated animals. CTX administrated 6 days before *iDex* vaccination was able to boost peptide specific secondary responses as assessed by immunomonitoring using specific fluorescent tetramers and IFNγ secretion by T cells after *in vitro* stimulation. Adoptive transfer of Treg curtailed CTX/*iDex* synergistic anti-tumor effects confirming the inhibiting role of Treg (Taïeb et al., 2006). Modulation of Dex bioactivity through modulation of DCs maturation stage was first described by Robbins' team in 2005 (Kim et al., 2005). This first study shows that Dex derived from DC treated with IL-10 are tolerogenic, resulting in the suppression of inflammation and collagen-induced arthritis in mice (Kim et al., 2005; Ruffner et al., 2009). Thereafter, this team and others have shown that the treatment of DCs with indoleamine 2,3-dioxygenase (IDO) (Bianco et al., 2009) or IL-4 (Kim et al., 2007a) but also the loading of alloantigens on DCs (Montecalvo et al., 2008; Pêche et al., 2003; Pêche et al., 2006) could lead to the production of tolerating Dex. Some molecules have been identified as responsible for this tolerance: MHC-II complexes (Kim et al., 2007b), Fas-L (Kim et al., 2006), CD80 and CD86 (Ruffner et al., 2009) (the two latter being weakly expressed on *iDex*). Conversely, it appears that exosomes secreted by mature DCs are immunogenic (mDex). Indeed, Dex produced from LPS-matured DC showed enrichment with ICAM-1 and CD86 leading to activation of $CD4^+$ and $CD8^+$ T cells (Segura et al., 2005b; Sprent, 2005). These studies highlighted the fact that Dex can be tailor-made *in vitro* for the treatment of autoimmune diseases, viral infection as well as cancer. In our group, we have engineered human mDex, respecting good laboratory processes (GLP), harboring a phenotype with increased expression of MHC complexes and costimulation molecules suggesting potent adaptive immunity (unpublished data). These second generation mDex are currently tested in a phase II clinical trial in non small cell lung cancer (NSCLC) bearing patients (Viaud et al., 2010).

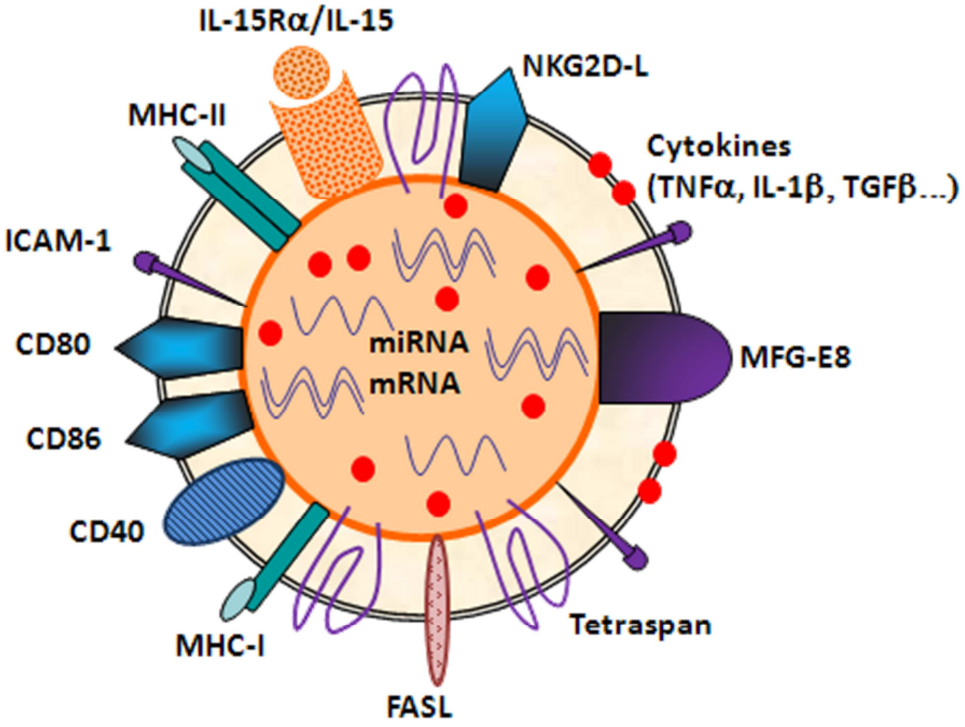

Figure 1. Molecules that influence Dex immunogenicity. Molecules that influence Dex immunogenicity. It is conceivable to think that the balance of expression of these markers could guide the immune response. Expression density of these molecules is modified according to the signals received by secreting DCs. Thus Dex enriched with antigen-loaded MHC-I,-II molecules, costimulatory molecules (CD40, CD80 and CD86), molecules that stimulate innate immunity (IL-15Rα/IL-15, NKG2D-L, proinflammatory cytokines) and molecules allowing the up-take by a DC subpopulation (ICAM-1) rather than by macrophages (MFG-E8) should induce potent adaptive and innate immune responses rather than ignorance or tolerance. Conversely DCs receiving anti-inflammatory signals (IL-10, TGFβ, IDO, IL-4 ...) can produce Dex poorly enriched with costimulatory molecules and ICAM-1 and expressing certain molecules that could directly inhibit adaptive immune responses (i, e. FASL) or stimulate tolerance. The biological role of mRNAs and microRNAs and their influence on Dex-mediated immune responses remain unknown. Finally, several molecules remain to be identified as well as the regulation of their expression on Dex. These latter points provide exciting lines of investigation for the future.

III) Molecules that regulate Dex immunogenicity (Fig. 1)

As mentioned above, modification of Dex molecular composition influences Dex's immune properties. Thus identification of molecules involved in immune responses generated by Dex seems paramount. Currently few molecules have been clearly identified. Some of them incline to immunogenicity: i) MHC molecules necessary to generate an adaptive immune response ii) CD54/ICAM-1 and mannose/glucosamine-rich C-type lectin receptor ligands (Hao et al., 2007; Segura et al., 2007) that could favor the uptake by endogenous DCs iii) B7.2/CD86 and CD54/ICAM-1 that could induce $CD4^+$ T cell responses (Segura et al., 2005b) or $CD8^+$ T cell responses (Sprent, 2005), iv) and B7.1/CD80 and B7.2/CD86 that could participate in DTH reactions (Ruffner et al., 2009) and v) IL-15Rα and NKG2D-L that promote the activation and proliferation of NK cells (Viaud et al., 2009). Other molecules, in contrast, promote tolerance: i) CD95 ligand/FASL probably in enhancing lymphocytes apoptosis (Kim et al., 2006), ii) MFG-E8/lactadherin (milk fat globule EGF factor VIII) highly expressed on *iDex* but weakly expressed on mDex (Segura et al., 2005a) could play a pivotal role in the immunogenicity of Dex. Indeed MFG-E8 (MFG-E8 binds to apoptotic cells, activated platelets, and phosphatidylserine-expressing cells via the C-domains and anchors them to macrophage integrins via its RGD sequence in the EGF domain) is involved in phagocytosis and destruction of apoptotic cells by macrophages (Hanayama et al., 2002) and could play a major role in maintaining peripheral tolerance (Asano et al., 2004; Hanayama et al., 2004). Thus highly MFG-E8-expressing exosomes could target dying cells and/or apoptotic bodies ensuring their clearance leading to a silent cell death for the immune system. Recently, some studies have suggested that *iDex* (enriched with MFG-E8) rescue septic animals via MFG-E8 thanks to a systemic inflammatory response decrease in sepsis by enhancing apoptotic cell clearance (Miksa et al., 2009; Miksa et al., 2006). By cons, mDEX expressing strongly ICAM-1 and weakly MFG-E8 could be mainly targeted on endogenous DCs and therefore be immunogenic (Segura et al., 2005a; Segura et al., 2005b; Segura et al., 2007). Besides these molecules that have been well studied during the development of an immune response induced by Dex, other mediators could participate. Some researchers have deliberately modified DCs to

make them express pro/anti-inflammatory cytokines leading to production of Dex with modified immune capacities (Dai et al., 2006). Recently, it has been shown that Dex could contain bioactive cytokines. One work suggested an alternative model of IL-1β release that may involve the P2X7R-induced formation of multivesicular bodies which contain exosomes with entrapped IL-1β and other inflammasome components (Qu et al., 2007). Moreover, C. Obregon and colleagues have demonstrated that Dex could represent important carriers of TNF-α (Obregon et al., 2009) highlighting the key role of DC-derived exovesicles, not only in adaptive immunity, but also in innate immunity by triggering innate immune responses and activating neighboring epithelial cells to release cytokines and chemokines, thereby amplifying the magnitude of the innate immune response. Finally, immunoregulation of Dex could also be controlled by their microRNAs content. Indeed, DC maturation signals lead to the induction or reduction of microRNAs' pool, some of them being pro-inflammatory while others anti-inflammatory (Lu and Liston, 2009; Rodriguez et al., 2007). Given that exosomes contain microRNAs that can be transferred to a target cell (Valadi et al., 2007) and knowing that secreted microRNAs could be mainly contained in exosomes (Kosaka et al., 2010) (exosomes being resistant to complement attack (Clayton et al., 2003) and protected from RNAses (Valadi et al., 2007)), it is conceivable that the microRNAs composition could influence the pro- or anti-inflammatory properties of Dex; however this hypothesis remains to be confirmed.

IV) Conclusion and perspectives

Although the full understanding of the significance of Dex requires additional studies, these vesicles proved to be a naturally occurring minimal antigen presenting unit that could orchestrate immune responses according to various signals received by producing DCs (Théry et al., 2009).

Exosomes are secreted in large quantities by DCs in the absence of any stimulation (steady state), in this context exosomes might play a role mainly in tissue homeostasis in promoting apoptotic body clearance and avoiding harmful inflammation that could lead to autoimmune diseases development. By cons, in an anti-inflammatory (IL-10, TGFβ, IDO ...) or a pro-inflammatory (LPS, Poly IC, IFNγ, ATP...) context, the molecular composition of Dex could play a fundamental role in shaping immune responses. Indeed, *in vivo* the targeting to one or the other exosomes presenting cell (MFG-E8, ICAM-1 ...), the expression of molecules that can regulate immunity (costimulatory molecules; FASL ...), the presence of inflammation mediators in Dex (IL-1β, TNFα, TGFβ1 ...) but also mRNAs or microRNAs content should dictate *in vivo* bioactivity of Dex. The role of Rab27a and Rab27b in regulating the secretion of exosomes has recently been identified (Ostrowski et al., 2010); this should enable us to build tools for the understanding of exosomes' role in different pathophysiological contexts. Our growing knowledge of the effects of Dex on immune responses and their potential use as therapeutic agents led us to develop second generation mDex in order to propose a maintenance immunotherapy in patients with advanced cancer (Fig. 2) (Viaud et al., 2010). During this phase II clinical trial, we will have a unique opportunity to analyze the immunostimulatory capacity of mDex in a dynamic study in patients, to investigate their protein and nucleic acid content and to correlate those parameters with a potential and expected clinical efficacy in patients. Together, these studies should allow us to identify molecules essential for the bioactivity of mDex *in vivo* in cancer patients.

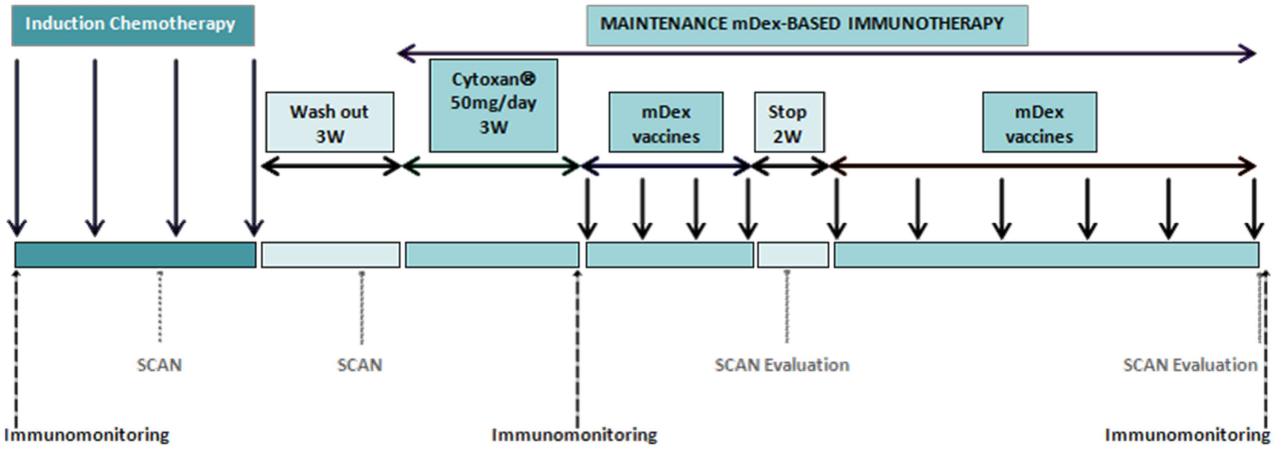

Figure 2. Phase II clinical trial using mDEX (Dex^2 trial). During this bicenter trial (Gustave Roussy and Curie Institutes, France), HLA-A2$^+$ patients suffering from inoperable NSCLC (Stage IIIB / IV) and responders to induction chemotherapy, will receive Dex-based maintenance immunotherapy (oral low dose cyclophosphamide for 3 weeks followed by intradermal vaccinations with mDex pulsed with tumor antigens). Clinical response evaluation after 4 and 6 vaccines will allow determining the efficacy of this therapeutic approach. In addition, numerous samples (blood and tumor) will allow building a link between mDex immunogenicity and possible clinical responses.

References

Zitvogel L, Regnault A, Lozier A, Wolfers J, Flament C, Tenza D, Ricciardi-Castagnoli P, Raposo G, Amigorena S. Eradication of established murine tumors using a novel cell-free vaccine: dendritic cell-derived exosomes. Nat Med. 1998 May;4(5):594-600

Hanayama R, Tanaka M, Miwa K, Shinohara A, Iwamatsu A, Nagata S. Identification of a factor that links apoptotic cells to phagocytes. Nature. 2002 May 9;417(6885):182-7

Lamparski HG, Metha-Damani A, Yao JY, Patel S, Hsu DH, Ruegg C, Le Pecq JB. Production and characterization of clinical grade exosomes derived from dendritic cells. J Immunol Methods. 2002 Dec 15;270(2):211-26

Théry C, Duban L, Segura E, Véron P, Lantz O, Amigorena S. Indirect activation of naïve CD4+ T cells by dendritic cell-derived exosomes. Nat Immunol. 2002 Dec;3(12):1156-62

Clayton A, Harris CL, Court J, Mason MD, Morgan BP. Antigen-presenting cell exosomes are protected from complement-mediated lysis by expression of CD55 and CD59. Eur J Immunol. 2003 Feb;33(2):522-31

Hsu DH, Paz P, Villaflor G, Rivas A, Mehta-Damani A, Angevin E, Zitvogel L, Le Pecq JB. Exosomes as a tumor vaccine: enhancing potency through direct loading of antigenic peptides. J Immunother. 2003 Sep-Oct;26(5):440-50

Pêche H, Heslan M, Usal C, Amigorena S, Cuturi MC. Presentation of donor major histocompatibility complex antigens by bone marrow dendritic cell-derived exosomes modulates allograft rejection. Transplantation. 2003 Nov 27;76(10):1503-10

André F, Chaput N, Schartz NE, Flament C, Aubert N, Bernard J, Lemonnier F, Raposo G, Escudier B, Hsu DH, Tursz T, Amigorena S, Angevin E, Zitvogel L. 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 Feb 15;172(4):2126-36

Asano K, Miwa M, Miwa K, Hanayama R, Nagase H, Nagata S, Tanaka M. Masking of phosphatidylserine inhibits apoptotic cell engulfment and induces autoantibody production in mice. J Exp Med. 2004 Aug 16;200(4):459-67

Chaput N, Schartz NE, André F, Taïeb J, Novault S, Bonnaventure P, Aubert N, Bernard J, Lemonnier F, Merad M, Adema G, Adams M, Ferrantini M, Carpentier AF, Escudier B, Tursz T, Angevin E, Zitvogel L. 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 Feb 15;172(4):2137-46

Ghiringhelli F, Larmonier N, Schmitt E, Parcellier A, Cathelin D, Garrido C, Chauffert B, Solary E, Bonnotte B, Martin F. CD4+CD25+ regulatory T cells suppress tumor immunity but are sensitive to cyclophosphamide which allows immunotherapy of established tumors to be curative. Eur J Immunol. 2004 Feb;34(2):336-44

Hanayama R, Tanaka M, Miyasaka K, Aozasa K, Koike M, Uchiyama Y, Nagata S. Autoimmune disease and impaired uptake of apoptotic cells in MFG-E8-deficient mice. Science. 2004 May 21;304(5674):1147-50

Escudier B, Dorval T, Chaput N, André F, Caby MP, Novault S, Flament C, Leboulaire C, Borg C, Amigorena S, Boccaccio C, Bonnerot C, Dhellin O, Movassagh M, Piperno S, Robert C, Serra V, Valente N, Le Pecq JB, Spatz A, Lantz O, Tursz T, Angevin E, Zitvogel L. Vaccination of metastatic melanoma patients with autologous dendritic cell (DC) derived-exosomes: results of thefirst phase I clinical trial. J Transl Med. 2005 Mar 2;3(1):10

Kim SH, Lechman ER, Bianco N, Menon R, Keravala A, Nash J, Mi Z, Watkins SC, Gambotto A, Robbins PD. Exosomes derived from IL-10-treated dendritic cells can suppress inflammation and collagen-induced arthritis. J Immunol. 2005 May 15;174(10):6440-8

Morse MA, Garst J, Osada T, Khan S, Hobeika A, Clay TM, Valente N, Shreeniwas R, Sutton MA, Delcayre A, Hsu DH, Le Pecq JB, Lyerly HK. A phase I study of dexosome immunotherapy in patients with advanced non-small cell lung cancer. J Transl Med. 2005 Feb 21;3(1):9

Segura E, Amigorena S, Théry C. Mature dendritic cells secrete exosomes with strong ability to induce antigen-specific effector immune responses. Blood Cells Mol Dis. 2005 Sep-Oct;35(2):89-93

Segura E, Nicco C, Lombard B, Véron P, Raposo G, Batteux F, Amigorena S, Théry C. ICAM-1 on exosomes from mature dendritic cells is critical for efficient naive T-cell priming. Blood. 2005 Jul 1;106(1):216-23

Sprent J. Direct stimulation of naïve T cells by antigen-presenting cell vesicles. Blood Cells Mol Dis. 2005 Jul-Aug;35(1):17-20

Chaput N, Flament C, Viaud S, Taieb J, Roux S, Spatz A, André F, LePecq JB, Boussac M, Garin J, Amigorena S, Théry C, Zitvogel L. Dendritic cell derived-exosomes: biology and clinical implementations. J Leukoc Biol. 2006 Sep;80(3):471-8

Dai S, Zhou X, Wang B, Wang Q, Fu Y, Chen T, Wan T, Yu Y, Cao X. Enhanced induction of dendritic cell maturation and HLA-A*0201-restricted CEA-specific CD8(+) CTL response by exosomes derived from IL-18 gene-modified CEA-positive tumor cells. J Mol Med. 2006 Dec;84(12):1067-76

Kim SH, Bianco N, Menon R, Lechman ER, Shufesky WJ, Morelli AE, Robbins PD. Exosomes derived from genetically modified DC expressing FasL are anti-inflammatory and immunosuppressive. Mol Ther. 2006 Feb;13(2):289-300

Miksa M, Wu R, Dong W, Das P, Yang D, Wang P. Dendritic cell-derived exosomes containing milk fat globule epidermal growth factor-factor VIII attenuate proinflammatory responses in sepsis. Shock. 2006 Jun;25(6):586-93

Pêche 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 Jul;6(7):1541-50

Taieb J, Chaput N, Schartz N, Roux S, Novault S, Ménard C, Ghiringhelli F, Terme M, Carpentier AF, Darrasse-Jèze G, Lemonnier F, Zitvogel L. Chemoimmunotherapy of tumors: cyclophosphamide synergizes with exosome based vaccines. J Immunol. 2006 Mar 1;176(5):2722-9

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

Ghiringhelli F, Menard C, Puig PE, Ladoire S, Roux S, Martin F, Solary E, Le Cesne A, Zitvogel L, Chauffert B. 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 May;56(5):641-8

Hao S, Bai O, Li F, Yuan J, Laferte S, Xiang J. Mature dendritic cells pulsed with exosomes stimulate efficient cytotoxic T-lymphocyte responses and antitumour immunity. Immunology. 2007 Jan;120(1):90-102

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 Aug 15;179(4):2242-9

Kim SH, Bianco NR, Shufesky WJ, Morelli AE, Robbins PD. MHC class II+ exosomes in plasma suppress inflammation in an antigen-specific and Fas ligand/Fas-dependent manner. J Immunol. 2007 Aug 15;179(4):2235-41

Qu Y, Franchi L, Nunez G, Dubyak GR. Nonclassical IL-1 beta secretion stimulated by P2X7 receptors is dependent on inflammasome activation and correlated with exosome release in murine macrophages. J Immunol. 2007 Aug 1;179(3):1913-25

Rodriguez A, Vigorito E, Clare S, Warren MV, Couttet P, Soond DR, van Dongen S, Grocock RJ, Das PP, Miska EA, Vetrie D, Okkenhaug K, Enright AJ, Dougan G, Turner M, Bradley A. Requirement of bic/microRNA-155 for normal immune function. Science. 2007 Apr 27;316(5824):608-11

Segura E, Guérin C, Hogg N, Amigorena S, Théry C. CD8+ dendritic cells use LFA-1 to capture MHC-peptide complexes from exosomes in vivo. J Immunol. 2007 Aug 1;179(3):1489-96

Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ, Lötvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol. 2007 Jun;9(6):654-9

Guo F, Chang CK, Fan HH, Nie XX, Ren YN, Liu YY, Zhao LH. Anti-tumour effects of exosomes in combination with cyclophosphamide and polyinosinic-polycytidylic acid. J Int Med Res. 2008 Nov-Dec;36(6):1342-53

Montecalvo A, Shufesky WJ, Stolz DB, Sullivan MG, Wang Z, Divito SJ, Papworth GD, Watkins SC, Robbins PD, Larregina AT, Morelli AE. Exosomes as a short-range mechanism to spread alloantigen between dendritic cells during T cell allorecognition. J Immunol. 2008 Mar 1;180(5):3081-90

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 Feb;60(2):380-9

Lu LF, Liston A. MicroRNA in the immune system, microRNA as an immune system. Immunology. 2009 Jul;127(3):291-8

Miksa M, Wu R, Dong W, Komura H, Amin D, Ji Y, Wang Z, Wang H, Ravikumar TS, Tracey KJ, Wang P. Immature dendritic cell-derived exosomes rescue septic animals via milk fat globule epidermal growth factor-factor VIII [corrected]. J Immunol. 2009 Nov 1;183(9):5983-90

Obregon C, Rothen-Rutishauser B, Gerber P, Gehr P, Nicod LP. Active uptake of dendritic cell-derived exovesicles by epithelial cells induces the release of inflammatory mediators through a TNF-alpha-mediated pathway. Am J Pathol. 2009 Aug;175(2):696-705

Qazi KR, Gehrmann U, Domange Jordö E, Karlsson MC, Gabrielsson S. Antigen-loaded exosomes alone induce Th1-type memory through a B-cell-dependent mechanism. Blood. 2009 Mar 19;113(12):2673-83

Ruffner MA, Kim SH, Bianco NR, Francisco LM, Sharpe AH, Robbins PD. B7-1/2, but not PD-L1/2 molecules, are required on IL-10-treated tolerogenic DC and DC-derived exosomes for in vivo function. Eur J Immunol. 2009 Nov;39(11):3084-90

Théry C, Ostrowski M, Segura E. Membrane vesicles as conveyors of immune responses. Nat Rev Immunol. 2009 Aug;9(8):581-93

Viaud S, Terme M, Flament C, Taieb J, André F, Novault S, Escudier B, Robert C, Caillat-Zucman S, Tursz T, Zitvogel L, Chaput N. Dendritic cell-derived exosomes promote natural killer cell activation and proliferation: a role for NKG2D ligands and IL-15Ralpha. PLoS One. 2009;4(3):e4942

Kosaka N, Iguchi H, Yoshioka Y, Takeshita F, Matsuki Y, Ochiya T. Secretory mechanisms and intercellular transfer of microRNAs in living cells. J Biol Chem. 2010 Jun 4;285(23):17442-52

Ostrowski M, Carmo NB, Krumeich S, Fanget I, Raposo G, Savina A, Moita CF, Schauer K, Hume AN, Freitas RP, Goud B, Benaroch P, Hacohen N, Fukuda M, Desnos C, Seabra MC, Darchen F, Amigorena S, Moita LF, Thery C. Rab27a and Rab27b control different steps of the exosome secretion pathway. Nat Cell Biol. 2010 Jan;12(1):19-30; sup pp 1-13

Viaud S, Théry C, Ploix S, Tursz T, Lapierre V, Lantz O, Zitvogel L, Chaput N. Dendritic cell-derived exosomes for cancer immunotherapy: what's next? Cancer Res. 2010 Feb 15;70(4):1281-5

This article should be referenced as such:

Viaud S, Desbois M, Ploix S, Zitvogel L, Chaput N. Natural nanoparticules against cancer: mature dendritic cell-derived exosomes. Atlas Genet Cytogenet Oncol Haematol. 2011; 15(2):230-235.