

CANCER IMMUNOTHERAPY USING DENDRITIC CELL-DERIVED EXOSOMES

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Abstract Dendritic cells (DCs) are the most potent antigen presenting cells and the only ones capable of inducing primary cytotoxic immune responses. We found that DCs secrete a population of membrane vesicles, called exosomes. Exosomes are 60-80 nm vesicles of endocytic origin. The protein composition of exosomes was subjected to a systematic proteomic analysis. Besides MHC and co-stimulatory molecules, exosomes bear several adhesion proteins, most likely involved in their specific targeting. We also found that exosomes accumulate several cytosolic factors, probably involved in their endosomal biogenesis. Like DCs, exosomes induce immune responses *in vivo*. Indeed, a single injection of DC-derived exosomes sensitized with tumor peptides induced potent anti tumor immune responses in mice and the eradication of established tumors. Tumor-specific cytotoxic T lymphocytes were found in the spleen of exosome-treated mice, and the anti tumor effect of exosomes was sensitive to *in vivo* depletion of CD8+ T cells. These results show that exosomes induce potent anti tumor effects *in vivo*, and strongly support the implementation of human DC-derived exosomes for cancer immunotherapy.

Key words: exosomes, immunotherapy, dendritic cells, cancer, antigen presentation

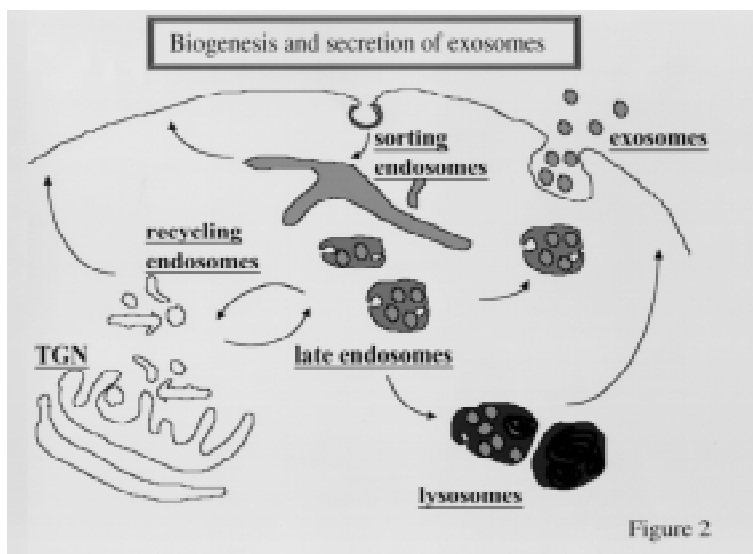
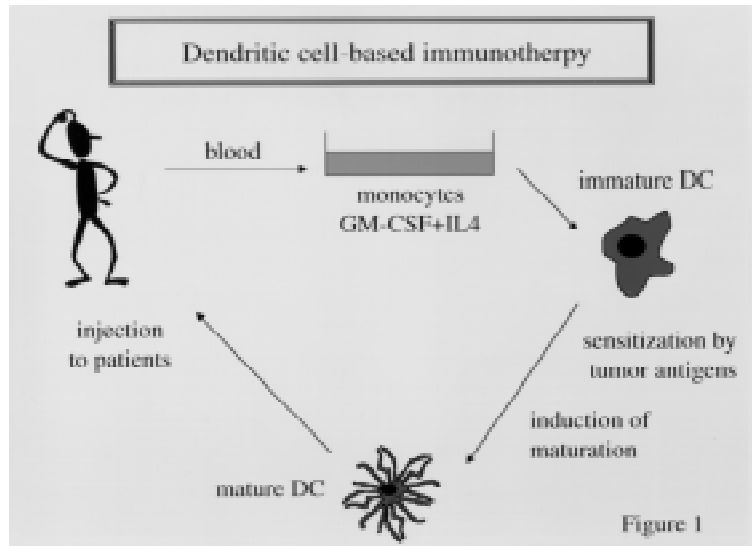
Resumen *Inmunoterapia en cáncer empleando exosomas derivados de células dendríticas.* De las células presentadoras de antígeno, las dendríticas (DC) son las más potentes y las únicas capaces de inducir una respuesta inmune citotóxica primaria. Hemos encontrado que las membranas de las DC segregan una población de vesículas, llamadas exosomas. Estos exosomas son vesículas de 60-80 nm de origen endosomal. Su composición proteica surgió de un análisis proteómico sistemático. Además de moléculas de MHC y otras co-estimuladoras, los exosomas engloban varias proteínas de adhesión, probablemente involucradas en su *targeting* específico. También encontramos que los exosomas acumulan factores citosólicos, probablemente relacionados con su biogénesis endosomal. Al igual que las DC, los exosomas inducen respuestas inmunes *in vivo*. De hecho, una simple inyección de exosomas derivados de DC sensibilizadas con péptidos tumorales indujo una fuerte respuesta inmune en ratones y la erradicación de tumores establecidos. Se encontraron linfocitos T citotóxicos tumor-específicos en el bazo de los ratones tratados con exosomas, y ese efecto anti-tumoral era abolido *in vivo* con la depleción de células CD8+. Estos resultados demuestran que los exosomas inducen una fuerte reacción anti-tumoral *in vivo*, abriendo el camino para la implementación de exosomas derivados de DC humanas en la inmunoterapia del cáncer.

Tumors express specific antigenic determinants, which may be recognized by the immune system and used as targets for immunotherapy¹. Different strategies for specific cancer immunotherapy were recently developed. One of the most promising approaches relies on the use of dendritic cells (DCs), the only antigen presenting cells capable of initiating immune responses through the activation of resting, naive T lymphocytes². The general scheme of this approach is shown in Figure 1. DCs are differentiated from the patient's blood monocytes by incubation in the presence of GM-CSF and IL-4. The DCs thus obtained are called immature, because they have high endocytic and phagocytic capacities and low T cell

stimulating activity. Immature DCs need to be sensitized with tumor antigens, which is generally achieved by using synthetic peptides derived from tumor antigen. Alternative sensitization methods developed recently, include the use of viral vectors, apoptotic tumor cells or RNA³.

After sensitization of DCs with tumor antigens, it is necessary to induce DC maturation to obtain T cell stimulatory DCs, before subcutaneous injection to the patients. Maturation is most often induced using a cocktail of cytokines, including TNF α . Several phase I clinical trials based on these schemes have been published recently^{4, 7}. They showed no toxicity (except several reported cases of vitiligo in melanoma trials). Objective immunological and clinical responses were obtained in around 25-30% of patients with lymphoma, melanoma and renal cell carcinoma.

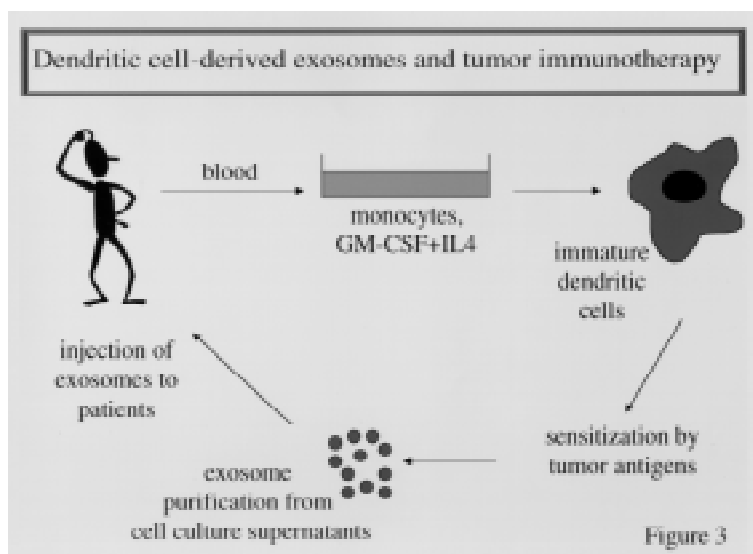
In the last few years, we have developed an alternative immunotherapy approach, based on the use of exosomes,



a population of membrane vesicles that may be compared to “natural liposomes”. We have shown, that like other cell types^{8, 9}, DCs produce and secrete exosomes. Exosome biogenesis occurs within a particular population of endosomes (called late endosomes or multivesicular endosomes) by invagination of the endosome limiting membrane towards the interior of the compartment (Fig. 2). After budding of the vesicles, they accumulate in the lumen of the endosome, until the complete compartment fuses with the plasma membrane, thus secreting the internal vesicles (which are then called exosomes) in the extracellular medium. Exosomes may be purified from

DC's culture supernatant by differential ultrafiltration and ultracentrifugation.

When produced by DCs that had been sensitized with tumor peptides, exosomes induced potent anti tumor immune responses and eradication of established tumors in mouse¹⁰. The effect of exosomes on tumor growth required T cells and induced immunological memory, as the mice that had eradicated their tumors were resistant to the establishment of the same tumor even after several months. Comparative studies showed that in the same tumor models, immunotherapy with exosomes was more effective using dendritic cells.



An extensive proteomic analysis of mouse DCs exosomes identified most of the major exosomal constituents, establishing a molecular map of this cellular compartment¹¹. Besides MHC and T cell co-stimulatory molecules, exosomes contain various cytosolic, endosome-related proteins, including Hsc70, Gi2 α and annexin II. These proteins are probably involved in exosome's intracellular biogenesis. Different membrane proteins involved in cell adhesion, like several integrins and CD9 accumulate in exosomes. In addition, and most surprisingly, a soluble milk fat globule protein, lactadherin, was also highly enriched in exosomes. These proteins are most likely responsible for exosome targeting to effector cells and biological activities.

Human dendritic cells also secrete exosomes, very similar in composition and morphology to murine exosomes. A process to purify human dendritic cell-derived exosomes for clinical use has been developed. A clinical phase I trial using exosomes produced by DCs sensitized with tumor antigen peptides, will start this year in France and the USA in melanoma and lung cancer patients (Fig. 3).

A second aspect of the biology of exosomes, concerns vesicles produced by tumor cells (Wolfers et al., submitted for publication). Tumor cell-derived exosomes share many of the markers found on dendritic cell-derived exosomes, as well as their morphological characteristics (size and shape). Exosomes purified from murine tumor cell lines were incubated with dendritic cells. Such sensitized dendritic cells vaccinated mice against the establishment of the same and of certain allogeneic tumors. Similarly, such dendritic cells also had an effect on established autologous or allogeneic tumors. Therefore, exosomes allowed cross-protection or cross-treatment in several murine models. When irradiated tu-

mor cells or tumor cell lysates were used, no anti tumor effects were observed. These anti tumor effects required T cells, demonstrating that tumor rejection was the result of a specific T cell response. Therefore, tumor exosomes contain all the "antigenic information" that dendritic cells need to efficiently trigger specific and efficient anti-tumor immune responses.

Using a human melanoma model, we also showed that tumor antigens may be processed and presented to specific T cells after melanoma exosomes were taken up by DCs. Exosomes from tumor cells contain tumor antigens, such as Melan A/MART-1 or tyrosinase, and heat shock proteins, such as hsc70.

We are currently considering the possibility to use tumor cell derived exosomes as a source of unidentified tumor antigens for DC sensitization in cancer immunotherapy.

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At long last, we may thus have the proper intellectual framework to understand most of the more common life-threatening cancers. At their essence are three types of genetic changes: 1) those that make cells divide when they should not, 2) those that lead to the formation of blood vessel that infiltrate into and thereby nourish the growing tumors, and 3) those that modify cell-recognizing molecules in ways leading to losses of their respective cell's ability to recognize their natural cellular partners. We have indeed come a very long way, but there remain myriad further details to unravel both currently known oncogenes and the many more oncogenes yet to be discovered. Many of these new observations will initially unsettle us and momentarily make us despair of ever being able to have a fair fight against an enemy that so constantly changes the face it presents. But now is most certainly not the moment to lose faith in our ability to triumph over the inherent complexity that underlies the existence of the living state. Given enough time, and the financial and moral resources that will let those born optimistic stay that way, the odds for eventual success in beating down cancer is on our side.

Después de mucho tiempo, tenemos por fin el marco intelectual apropiado para entender los cánceres con más alta mortalidad. Esencialmente los cambios genéticos son de tres tipos: 1) los que llevan a las células a dividirse cuando no lo tendrían que hacer, 2) los que llevan a la formación de vasos sanguíneos que infiltran y nutren los tumores en desarrollo, 3) los que modifican las moléculas asociadas al reconocimiento celular llevando a la pérdida de la habilidad de la célula para reconocer sus *partenaires* naturales. No hay duda que hemos recorrido un largo camino, pero quedan miríadas de detalles para develar el funcionamiento tanto de los oncogenes ya conocidos como de los muchos todavía por descubrir. Muchas de estas nuevas observaciones inicialmente nos desconcertarán y momentáneamente nos harán desesperar de poder vencer un enemigo que constantemente cambia de cara. Sin embargo, ahora no es ciertamente el momento de perder la fe en nuestra habilidad de triunfar sobre la inherente complejidad que forma la base del estado viviente. Dado suficiente tiempo, y los recursos financieros y morales necesarios para que los optimistas de nacimiento sigan siéndolo, las probabilidades para un éxito eventual en vencer al cáncer siguen a favor nuestro.

James D. Watson

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