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Phenotype and functional analysis of human monocytes-derived dendritic cells loaded with a carbosilane dendrimer

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ABSTRACT

Dendritic cells (DCs) play a major role in development of cell-mediated immunotherapy due to their unique role in linking innate and adaptive immunities. In spite of improvement in this area, strategies employing *ex vivo* generated DCs have shown limited efficacy in clinical trials. Dendrimers have been proposed as new carriers for drug delivery in aim to ameliorate DCs antigen loading that is a pivotal point in DCs approaches. In this study, we have investigated the phenotypic and functional characteristics of human monocytes-derived dendritic cells after HIV-derived peptides uptake *in vitro*. We have found that iDCs and mDCs were able to capture efficiently water soluble carbosilane (CBS) dendrimer 2 G-NN16 and did not induce changes in maturation markers levels at the DCs surface. Therefore, CBS 2 G-NN16-loaded mDCs migrated as efficiently as unloaded DCs towards CCL19 or CCL21. Furthermore, DCs viability, activation of allogenic naïve CD4 + T cells by mDCs and secretion of cytokines were not significantly changed by 2 G-NN16 loading. Summing up, our data indicate that CBS 2 G-NN16 has no negative effects on the pivotal properties of DCs *in vitro*. It should therefore be feasible to further develop this antigen loading strategy for clinical use in immunotherapy against viral infections.

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1. Introduction

The present anti-HIV vaccine candidates have failed to protect from HIV replication in the long term and from immune system exhaustion associated to HIV infection. In the past few years a lot of difficulties have raised in development of new therapeutic vaccine candidates [1,2]. Moreover, the constraint of active antiretroviral therapy (ART) limitation has led to the development of immunotherapies (also known as immunovaccines), notably based on dendritic cells (DCs) to enhance anti-HIV immune responses during ART. The immunostimulatory potential of DCs has led to their exploration as cellular vaccines or adjuvant in immunotherapies against cancer or viral infections such as HIV infection [3–8]. Adequately activated myeloid DCs regulate important functions during HIV infection such as antibodies mediating neutralization, cytotoxicity, complement dependent lyses and other antiviral activities.

Up to now, clinical trials using these DC-based vaccines have shown that the rates of objective clinical benefit are low in vaccine recipients for cancer and in HIV trials [5,9,10]. However, several studies employing *ex vivo* generated DCs have shown a correlation between the persistence of antigen loaded in DCs and the magnitude of the immune responses [5,9,11]. Therefore, the choice of activation and mode of DCs loading are critical for the immune responses orientation. An inaccurate activation or maturation of

Abbreviations: mAbs, monoclonal antibody; ART, active antiretroviral therapy; CBS, carbosilane; CFSE, carboxyfluorescein succinimidyl ester; CCL19 or CCL21, C–C motif chemokine 19 or 21; MHC-II, major histocompatibility complex class II; iDC or mDC, immature or mature dendritic cells; GM-CSF, granulocyte-macrophage colony stimulating Factor; HIV, human immunodeficiency virus; HLA-DR, human leukocyte antigen DR; IFN, interferon; IL, interleukin; LDH, *Lactate dehydrogenase*; LPS, lipopolysaccharide; PBMC, peripheral blood mononuclear cells; PHA, phytohemagglutinin; poli(I:C), polyinosinic polycytidylic acid; PI, propidium iodide; PGE2, prostaglandin 2; NMR spectroscopy, nuclear magnetic resonance spectroscopy; Th1 or Th2, T cells helper 1 or 2; TNF, *Tumor necrosis factor*.

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these cells could induce an anergy of immune system instead of specific stimulation [12]. Therefore, a complete evaluation of the efficacy of *ex vivo* DC therapy must be driven by: a number of injected DCs, a method of antigen loading in DCs, a route of administration, the methods of immune monitoring and study design. Finally, the central difficulty of anti-HIV DC-based system is the capture and processing of HIV-derived proteins or peptides used as antigens in the way to activate specifically naïves or memories CD4 + T and CD8 + T cells.

Recently, synthetic molecules have been proved to be highly efficient to transduce various types of cells in less invasive and potentially more harmless approaches. High numbers of studies have been focused on dendrimers as carriers of drugs and biomolecules [13–17]. Dendrimers are chemical versatile polymers with definite forms, nanoscopic size and with physico-chemical properties identical to biomolecules. These particles are highly branched having multivalent functional groups, which increase their action in a synergic way. Dendrimers are molecules with a structure highly defined and with low-level of polydispersion in comparison to traditional polymers [18]. Carbosilane dendrimers (CBS) have been recently designed and analysed by ourselves [13,19]. These dendrimers are water soluble and highly biocompatible with cell lines and primary cells such as peripheral blood mononuclear cells (PBMCs). Moreover, CBS alone are not able to induce PBMC proliferation in comparison with mitogenic stimuli such as phytohemagglutinin (PHA) [13,19]. Finally, the interaction between CBS and biomolecules is able to preserve the conformation of the biomolecules that is essential for further drug or peptides functionality [19,20].

We proposed to test the 2 G-NN16 dendrimer ability to transport biomolecules as HIV-derived peptides into human monocytes-derived DCs. Our first objective was to determine if 2 G-NN16 in association with HIV-derived peptide was able to increase the uptake of these peptides into DCs in comparison to HIV-derived peptides alone. Our second objective was to show if DCs treated with CBS dendrimer associated to HIV-derived peptides are fully functional with respect to maturation, viability, migration, cytokines production and T cell allostimulation. These two objectives are equally essential to determine if 2 G-NN16 could be used as a tool as cellular vaccine or adjuvant for HIV immunotherapy in future.

2. Materials and methods

2.1. Materials and cell reagents

Three different HIV-derived peptides were synthesized (Eurogentec, Sereing, Belgium) and labelled with fluorescein; peptide derived from Nef sequence; HIV-HXB2 location Nef (172-191): NH-GMDDPEREVLEWRFDSRLAF-COOH (length 20 amino-acids, charged (−3)); peptide derived from Gag-P24 sequence; HIV-HXB2 location P24 (71–80): NH-DTINEEAAEW-COOH (length 10 amino-acids, charged (−4)) and peptide derived from envelop Gp160 (HIV-HXB2 location Gp160 (634–648): NH-EIDNYTNTIYTLLEE- COOH (length 15 amino-acids, charged (−4)). Whereas these peptides were only part of proteins, names of complete proteins were used to distinguish peptides used in experiments as Nef, Gp160 and P24 peptides.

2.2. Preparation of carbosilane dendrimer

Carbosilane dendrimer (CBS) was synthesized as previously reported [19] (see Fig. 1). The method of preparation of the dendrimers follows a divergent procedure. The dendrimer 2 G-CBS-$(OCH_2CH_2N^+Me_2CH_2CH_2N^+Me_3I^-)_8$ also known as 2 G-NN16 was used in all experiments, indicating that it is a second generation of dendrimer based on the number of positive charges each one possesses. At pH 7.4 (physiological pH), 2 G-CBS-dendrimer possess 16 positive charges as has been shown by ^{1}H NMR and ^{13}C NMR spectroscopy measurements [19]. Dendriplex refers to the association of 2 G-NN16 with HIV-derived peptides. All experiments were performed with 2.5 μM of 2 G-NN16 and 1 μM of peptides. Dendriplexes were formed by mixing HIV-derived peptides and dendrimer dissolved in RPMI 1640 medium (Sigma, St-Louis, MO, USA) free of serum at concentrations depending on the ± charge ratio (± ratio 10) with an incubation time of 30 min at 37 °C.

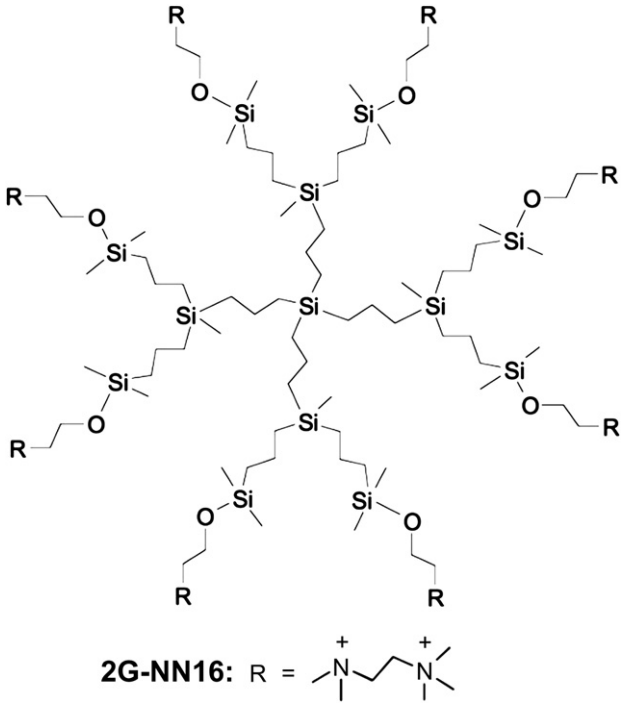

Fig. 1. Structure of carbosilane dendrimer (2 G-NN16).

2.3. Generation of dendritic cells

Human peripheral blood mononuclear cells (PBMCs) were obtained from healthy volunteers with Lymphocytes Isolation Solution gradient (Rafer, Madrid, Spain) [21]. Monocytes were separated with anti-human CD14 monoclonal antibody (mAb)-coated MicroBeads using MACS single-use separation columns (Miltenyi Biotec, Bergisch Gladbach, Germany). Monocytes obtained were plated in a flat-bottomed 6-well tissue culture plate (2×10^6/well) and cultured with DC-medium: RPMI 1640 medium containing 10% heat-inactivated foetal calf serum, 100 U/ml penicillin G, 100 μg/ml streptomycin (Sigma), 50 ng/ml rh GM-CSF, 20 ng/ml rh IL-4 (ImmunoTools, Friesoythe, Germany) and 50 nM of β-2-mercaptoethanol (Sigma). Fresh culture medium containing rh GM-CSF and rh IL-4 was added to immature DC (iDC) culture every 2 days. iDCs were matured by 20 ng/ml LPS (Sigma) for the last 2 days. Alternatively, iDCs were matured with 2 μg/ml Polyinosinic polycytidylic acid = poly(I:C) (Alexis Biochemicals, Farmingdale, NY, USA) or by a cytokines mix; 20 ng/ml TNFα (Miltenyi Biotech), 10 ng/ml IL-1β (Miltenyi Biotech) and 1 μg/ml Prostaglandin E2 (Sigma).

2.4. Determination of dendritic cells phagocytosis

iDCs or mature DC (mDCs) ($0{,}25 \times 10^6$ cells into 24-well plates) were loaded with peptides, 2 G-NN16 or dendriplexes (2 G-NN16/

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