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Short communication

Engineered exosomes boost the HCV NS3-specific CD8$^+$ T lymphocyte immunity in humans

Simona Anticoli [a], Emiliana Falcone [b], Anna Ruggieri [b], Maurizio Federico [a,*]

[a] National AIDS Center, Istituto Superiore di Sanità, Viale Regina Elena, 299, 00161 Rome, Italy
[b] Viral Zoonosis Unit, Department of Veterinary Public Health & Food Safety, Istituto Superiore di Sanità, Viale Regina Elena, 299, 00161 Rome, Italy

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ABSTRACT

At the present, no anti-Hepatitis C virus (HCV) HCV vaccine is available, and many patients failed the treatment with new class of HCV inhibitors. In HCV infection, both experimental and clinic evidences indicate that a strong CTL-immune response could have significant therapeutic effects. We developed an innovative anti-HCV CD8$^+$ T immunogen based on the uploading in engineered exosomes of full-length HCV-NS3 protein. HCV NS3 exosomes appeared immunogenic when injected in mice, as proven by the detection of a memory CD8$^+$ T lymphocyte pool two weeks after the last of three immunizations. On the other hand, dendritic cells isolated from PBMCs of HCV infected patients activate autologous HCV NS3-specific CD8$^+$ T lymphocytes upon challenge with HCV NS3 exosomes. These results provide the proof-of-principle that engineered exosomes can boost the CD8$^+$ T cell immunity in HCV-infected patients, thus representing a suitable option for patients resisting the therapies with recently discovered HCV inhibitors.

1. Introduction

Although an effective new class of HCV NS3/NS4A and NS5B inhibitors (i.e., boceprevir and sofosbuvir) is available [1], however drug resistance, suboptimal activity against diverse HCV genotypes, and their extremely high cost limit the expectancy of HCV eradication, while prompting the research for innovative vaccine strategies [2]. A both strong and broad CTL immune response targeting cells expressing HCV antigens is expected to be a key step towards viral clearance. In fact, the presence of multispecific IFN-γ producing, HCV-specific CD8$^+$ T cells have been reproducibly detected in infected hosts recovering from acute HCV infection [3,4]. Consistently, in chronic HCV infection the functionality of HCV-specific CD8$^+$ T cells is significantly altered [5].

Exosomes are vesicles of 50–100 nm released constitutively by all cell types [6]. They form intracellularly upon inward invagination of endosome membranes. Leading to the formation of intraluminal vesicles which thereby form multivesicular bodies. They can traffic either to lysosome vesicles or to the plasma membrane thereby releasing their vesicular contents in the extra-cellular milieu upon fusion with plasma membrane. Vesicles released by this mechanism are defined exosomes.

Exosomes are nanoparticles having a low intrinsic immunogenic profile. Their immunogenicity is basically related to the amounts and quality of antigens they incorporate. Exosomes have been tested in a number of clinical trials demonstrating both feasibility and good tolerance of exosomes as cell-free vaccines. However, the therapeutic efficacy appeared quite limited posing the need for new methods to increase their immunogenicity.

Engineering exosomes to upload heterologous proteins represents the last frontier in terms of nanoparticle-based technology. We optimized a method of protein incorporation in exosomes by exploiting the unique properties of a non-functional mutant of the HIV-1 Nef protein referred to as Nef^{mut}. It incorporates at high extents in exosomes meanwhile acting as carrier of protein antigens fused at its C-terminus [7]. In this way, the Nef^{mut}-based fusion products remain protected from external behavior. These features, together with the flexibility in terms of incorporation of foreign antigens and ease of production, make Nef^{mut}-based exosomes a convenient vehicle for immunogens.

Abbreviations: HCV, Human hepatitis C virus; CTLs, cytotoxic T lymphocytes; kDa, kilodaltons; VSV-G, vesicular stomatitis virus G protein; PBMCs, peripheral blood mononuclear cells; FACS, fluorescence-activated cell sorting; HPV, Human Papilloma Virus; FCS, fetal calf serum; APCs, antigen-presenting cells; CTL, cytotoxic T lymphocytes; TAA, tumor-associated antigens; iDCs, immature dendritic cells; AchE, acetylcholinesterase; PMA, phorbol 12 myristate 13-acetate; SD, standard deviation; SFU, spot-forming units; ALT, alanine transaminase.

* Corresponding author.
E-mail addresses: simona.anticoli@iss.it (S. Anticoli), emiliana.falcone@iss.it (E. Falcone), anna.ruggieri@iss.it (A. Ruggieri), maurizio.federico@iss.it (M. Federico).

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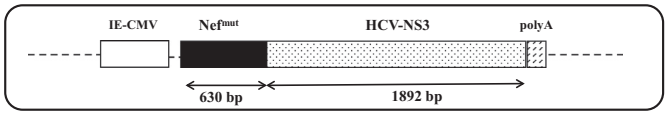

Fig. 1. Scheme of the pcDNA3-based vector expressing the Nef^{mut}/NS3 fusion protein. The positions of promoter (IE-CMV), Nef^{mut}/HCV-NS3 fused open reading frame, and poly A signal are signed. Base pair lengths of each moiety are also indicated.

HIV-1 Nef is a 27 kDa protein lacking enzymatic activities, however acting as a scaffold/adaptor element [8]. Nef^{mut} lacks basically all Nef functions [9], whereas incorporating in exosomes up to 100-fold most efficiently than the wild-type isoform. Recently, we showed that a viral TAA uploaded in Nef^{mut}-based engineered exosomes is cross-presented in targeted APCs, thereby eliciting a strong CTL immunity in mice which associates with clearing of syngeneic tumor cells implanted after immunization [10]. In the perspective to apply our previous findings to the treatment of HCV infection, engineered exosomes uploading HCV NS3 were pro-

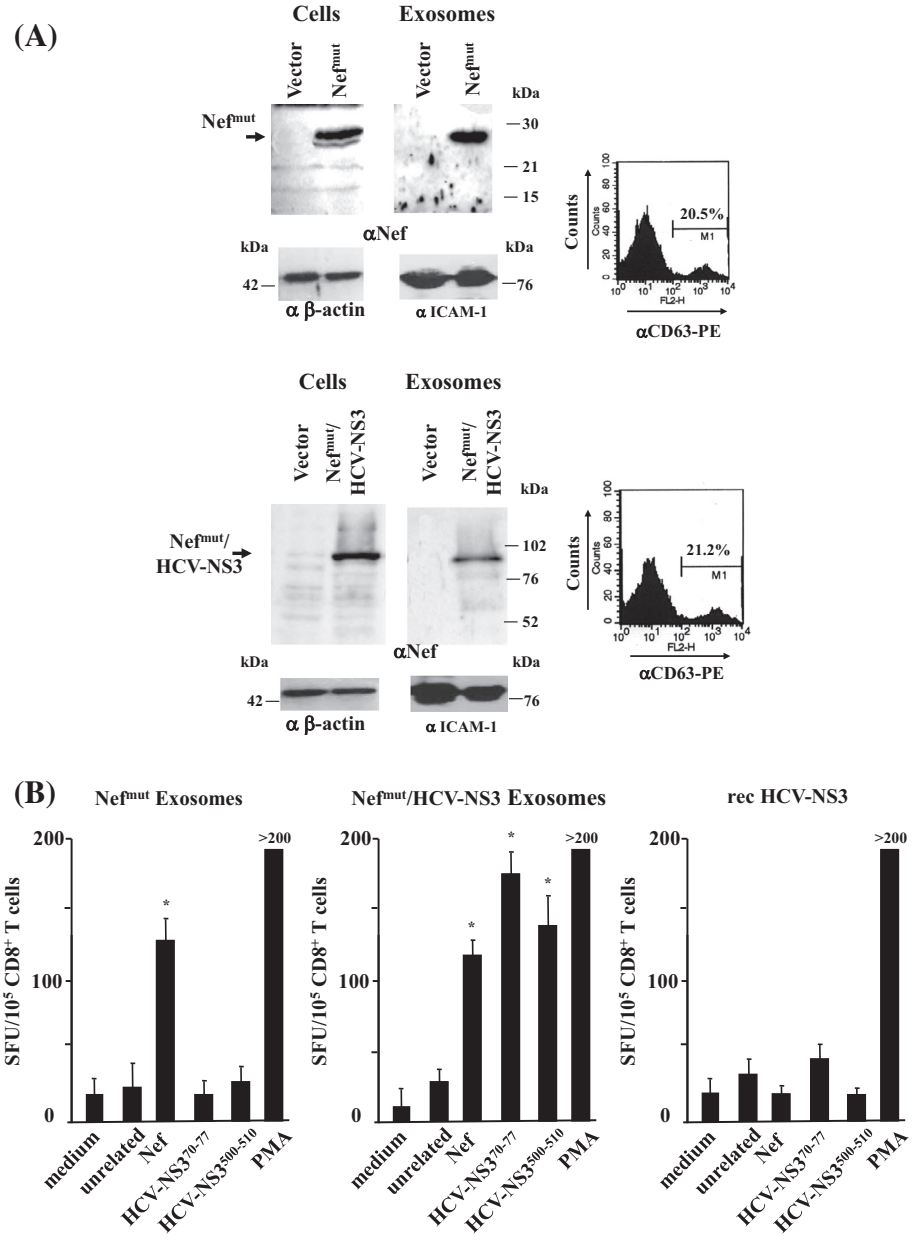

Fig. 2. HCV NS3-specific immunity induced in mice by inoculation of Nef^{mut}/NS3 exosomes. (A) Molecular characterization of exosome preparations uploading either Nef^{mut} (upper panels) or Nef^{mut}/NS3 (lower panels). A total of 30 μg (for cell lysates) and 200 μU equivalent of AchE activity of exosomes were assayed in western blot assays probed with anti-Nef, anti-β-actin (for cell lysates only), and anti-ICAM-1 (for exosomes only) antibodies. Arrows sign the relevant protein products. Molecular markers are given in kDa. In addition, both exosome preparations were tested for CD63 contents (right panels). Quadrants were set on the basis of the fluorescence of beads alone incubated with anti-CD63 mAb. Percentages of positive events are indicated. Results are representative of the assays performed on three different exosome preparations. (B) CD8$^+$ T cell immune response in mice inoculated with either Nef^{mut}-, Nef^{mut}/NS3 exosomes, or recombinant NS3 protein. C57Bl/6 mice (5 per group) were inoculated three times and, two weeks after the last inoculation, splenocytes were isolated and incubated 5 days in the presence or not of 5 μg/ml of either unrelated, Nef-, or NS3-specific peptides. Afterwards, cell activation extents were evaluated by IFN-γ Elispot assay carried out in triplicate with 10^5 cells/well. As a control, untreated cells were also incubated with 5 ng/ml of PMA and 500 ng/ml of ionomycin. Shown are the mean + SD number of SFU/10^5 cells. $^*p < 0.05$. The paired Student's *t*-test was used and confirmed using the non-parametric Wilcoxon rank sum test.