

Original article

A vaccine based on exosomes secreted by a dendritic cell line confers protection against *T. gondii* infection in syngeneic and allogeneic mice

Céline Beauvillain^{a,b,c,*}, Sophie Ruiz^{a,1}, Rachel Guiton^a,
Daniel Bout^a, Isabelle Dimier-Poisson^a

^a Université François-Rabelais, INRA, UMR 0483 Université-INRA d'Immunologie Parasitaire et Vaccinologie,

IFR des Agents Transmissibles et Infectiologie, UFR des Sciences Pharmaceutiques, 31, Avenue Monge, 37200 Tours, France

^b Unité Mixte Institut National de la Santé et de la Recherche Médicale 564, University Hospital, 4, rue Larrey, 49933 Angers, France

^c Immunology and Allergy Laboratory, University Hospital, Angers, France

Received 20 October 2006; accepted 7 July 2007

Available online 15 July 2007

Abstract

Our results show that exosomes secreted by SRDC pulsed *in vitro* with *Toxoplasma gondii*-derived antigens (Exo-TAg) induced protective responses against infection with the parasite in both syngeneic and allogeneic mice. After oral infection, syngeneic CBA/J mice exhibited significantly fewer cysts in their brains and allogeneic C57BL/6 mice survived. This protection was associated with strong humoral responses *in vivo* in serum from both CBA/J and C57BL/6 mice, and with high levels of anti-TAg IgA antibodies in intestinal secretions from CBA/J mice alone. Furthermore, strong cellular responses *in vivo* were observed in both mouse models. Cellular proliferation was associated with cytokines production by spleen and mesenteric lymph node cells. The results presented here show that exosomes are nucleic acid free vesicles that are able to induce immune responses correlated with protection against parasitic infections in both syngeneic and allogeneic mice. They could constitute an efficient tool for use in vaccination and antitumor strategies based on exosomes.

© 2007 Elsevier Masson SAS. All rights reserved.

Keywords: Exosomes; Dendritic cells; *Toxoplasma gondii*; Vaccine

1. Introduction

The coccidian protozoan *Toxoplasma gondii* (*T. gondii*) is an obligate intracellular parasite responsible for toxoplasmosis [1]. After a primary infection in immunocompetent individuals, the replication of tachyzoites is limited, resulting in the formation of bradyzoite forms, a dormant stage of the parasite. Usually asymptomatic, toxoplasmosis can lead to severe damage if associated with immunosuppression due to the

reactivation of encysted parasites or transmission to the fetus during pregnancy. Several studies aim to identify protective anti-*T. gondii* vaccine. Attenuated *T. gondii* tachyzoite vaccines have been successfully used for animal use [2], but vaccination with live organisms cannot be safely performed in humans. Protective antigens were identified among parasite surface molecules such as SAG1 [3] and induced the development of significant but partial protection in animal models.

Dendritic cells (DCs) are professional antigen-presenting cells that control the outcome of the immune response [4]. Due to these properties, DCs have been proposed as vaccine vectors [5]. We previously reported that transfer of DCs pulsed with *T. gondii* antigens (TAg) to healthy mice induced protection against a virulent oral challenge of *T. gondii* [6]. However, this approach is limited due to difficulty to obtain high quantity of DCs suitable for vaccination [7].

* Corresponding author at: Unité Mixte Institut National de la Santé et de la Recherche Médicale 564, University Hospital, 4, rue Larrey, 49933 Angers, France. Tel.: +33 (0)2 4135 4733; fax: +33 (0)2 4173 1630.

E-mail address: celine.beauvillain@univ-angers.fr (C. Beauvillain).

¹ Authors contribute equally to this work. CB is a fellow from Ministère de l'Éducation Nationale et de la Recherche.

The ability of exosomes, especially those derived from DCs, to induce protective immune responses offers an alternative to DC-based vaccines. Exosomes are cell-free membrane vesicles and secreted by several cell types [8–10]. Exosomes display functional MHC class I and class II and T-cell costimulatory molecules on their surface [8]. Exosomes derived from the DC cell line D1 and loaded with tumor peptides induce a protective immune response, leading to the eradication of established tumors in mice [11]. The potential of exosome-based vaccines was evidenced in a Phase I clinical trial in stage IV melanoma patients (long-term safety and *in vivo* bioactivity) [12].

We recently reported that SRDC (CD8 α + CD4– DC cell line) elicit protection in syngeneic mice against *T. gondii* infection [13]. In this study, we evaluated whether SRDC-derived exosomes may induce a protective immune response against *T. gondii* in syngeneic CBA/J mice and in allogeneic C57BL/6 mice.

2. Materials and methods

2.1. Mice

All *in-vivo* immunization experiments were carried out using female CBA/J (H-2^k) and C57BL/6 (H-2^b) mice (Janvier, France) and maintained in a pathogen-free environment. CBA/J mice are resistant to the acute phase of infection by *T. gondii* resulting in brain cyst formation whereas C57BL/6 mice are sensitive and die 8–10 days after the oral challenge.

2.2. Parasites

Tachyzoites of the RH strain of *T. gondii* were harvested from the peritoneal fluid of Swiss OF1 mice, intraperitoneally infected 3–4 days earlier. Tachyzoites were washed with RPMI 1640 medium (Invitrogen), sonicated and centrifuged at 2000 $\times g$ for 30 min at 4 °C. The supernatant from the last centrifugation was concentrated through dialysis tubing to produce aliquots. A protein assay reagent kit (MicroBCA; Sigma-Aldrich, St-Louis, USA) was used to determine the protein concentration. Cysts of the *T. gondii* 76K strain were obtained from the brain of orally infected CBA/J mice with 80 cysts one month earlier.

2.3. Exosome purification

The previously described [13], DC cell line SRDC was cultured for 18 h without or with 25 $\mu\text{g ml}^{-1}$ of TAG. The supernatants were recovered and centrifuged twice for 30 min at 4 °C, at 3000 and 10,000 $\times g$, respectively. Exosomes were pelleted at 100,000 $\times g$ for 1 h at 4 °C, washed in PBS and ultracentrifuged again. After resuspension, the protein concentration was determined using micro-BCA kit. A total of 10 μl of exosome solution was used for microscopy observation, made with a Jeol 1010 XC electron microscope (Tokyo, Japan).

2.4. Quantitation of *Toxoplasma* antigen in the preparations of exosomes

The content of free TAG into the purified exosomes was determined by competitive inhibition ELISA. Briefly, wells coated with 5 $\mu\text{g ml}^{-1}$ TAG, and blocked with 2% BSA, were incubated with serial 2-fold dilution of purified TAG standard or TAG-exosomes diluted in a 1/100 dilution of mouse anti-toxoplasma sera. Control-exosomes were used as negative controls. The Ig binding was detected with a goat anti-mouse IgG alkaline phosphatase conjugate (Sigma) diluted 1:5000 in BSA 4%. The limit of detection of the assay was 5 ng ml^{-1} TAG.

2.5. Adoptive immunizations and *Toxoplasma* challenge

Exosomes isolated from unpulsed-SRDC (Exo-NP) or TAG-pulsed-SRDC (Exo-TAG) supernatants were purified as previously described. Mice (12 by groups) received intravenously 5 μg of Exo-NP, Exo-TAG, TAG or 5 μg of Exo-NP co-injected with 5 μg of TAG (Exo-NP + TAG) in 200 μl of PBS at day 0 and day 15. CBA/J and C57BL/6 mice were orally challenged with 80 cysts and 30 cysts of *T. gondii* 76K strain, respectively, at day 30. Serum, intestinal secretions, spleen and mesenteric lymph nodes were harvested 10–13 days after each passive exosome transfer. At day 30 post-oral challenge CBA/J mice were sacrificed and each brains were recovered separately for cerebral cyst counting. Results are expressed as cyst number (mean $\pm$ SD). For the C57BL/6 mice, the mortality was followed daily from day 9 to 21 after the oral challenge.

2.6. Analysis of humoral and cellular immune response

Serums and intestinal secretions were recovered and stored at –20 °C. Immunoblotting was performed as previously described, using TAG [14]. Serum and intestinal secretions from infected mice and 1E5 mAb (anti-SAG1) were used as positive controls. For lymphocytes proliferation and cytokine analysis, spleen and MLN cells from CBA/J and C57BL/6 mice were seeded in triplicate in 96-well culture plates (5 $\times 10^5$ cells/well) and cultured 3 days in supplemented RPMI medium without or with 2–10 $\mu\text{g ml}^{-1}$ TAG. Cells were pulsed with 0.5 μCi ³H-thymidine per well for 18 h. Radioactivity was measured by liquid scintillation counting. Results are expressed as a stimulation index defined as follows: A/B where A and B are the cpm values obtained with TAG-stimulated and non-stimulated cells, respectively. Culture supernatants were harvested at different times, from 24 h to 96 h and assayed for IL-2, IL-4, IL-10, and IFN- γ production using commercial ELISA kits following to the manufacturer's instructions (BD pharmingen, San Diego, California, USA).

2.7. Short-term homing

Freshly-purified exosomes from SRDC-cells were labeled with 1 μCi of ⁵¹Cr per μg of exosomes for 2 h at 37 °C and washed by successive centrifugations. Exosomes (10 μg) were injected into the tail vein of CBA/J and C57BL/6 mice.

Download English Version:

<https://daneshyari.com/en/article/3416017>

Download Persian Version:

<https://daneshyari.com/article/3416017>

[Daneshyari.com](https://daneshyari.com)