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Vaccination with *Leishmania* histone H1-pulsed dendritic cells confers protection in murine visceral leishmaniasis

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ABSTRACT

Visceral leishmaniasis is the most severe form of leishmaniases affecting millions of people worldwide often resulting in death despite optimal therapy. Thus, there is an urgent need for the development of effective anti-infective vaccine(s). In the present study, we evaluated the prophylactic value of bone marrow-derived dendritic cells (BM-DCs) pulsed with the *Leishmania* (L.) *infantum* histone H1. We developed fully mature BM-DCs characterized by enhanced capacity of IL-12 production after ex vivo pulsing with GST-LeishH1. Intravenous administration of these BM-DCs in naive BALB/c mice resulted in antigen-specific spleenocyte proliferation and IgG1 isotype antibody production and conferred protection against experimental challenge with *L. infantum* independently of CpG oligonucleotides (ODNs) co-administration. Protection was associated with a pronounced enhancement of parasite-specific IFNγ-producing cells and reduction of cells producing IL-10, whereas IL-4 production was comparable in protected and non-protected mice. The polarization of immune responses to Th1 type was further confirmed by the elevation of parasite-specific IgG2a/IgG1 ratio in protected mice. The above data indicate the immunostimulatory capacity of *Leishmania* histone H1 and further support its exploitation as a candidate protein for vaccine development against leishmaniasis.

1. Introduction

Visceral leishmaniasis (VL) is the most severe form of leishmaniases, caused by the protozoan parasites *L. infantum/chagasi* and *L. donovani*. The existing antiparasitic treatment usually leads to severe side effects and establishment of resistant parasites. Therefore, the use of a vaccine could be an efficacious method for the control of VL [1]. Since control of leishmanial infection is mediated by Th1-type immune responses and IFNγ production [2,3], vaccine development towards *Leishmania* infection has been focusing on the identification and use of define recombinant antigens able to elicit protective T cell responses.

In recent years, two independent studies involved in screening of *L. donovani* expression cDNA library [4] and parasite-specific T cell lines [5] came up with histone proteins as strong potential vaccine candidates. Nucleosomal histone proteins (H2A, H2B, H3, and H4) in conjunction with DNA form nucleosomes that are linked by histone H1. *Leishmania* histone H1 possess homology with the higher eukaryotic histone H1 [6–8] and is associated with parasite infectivity [9].

Leishmania infection is correlated with significant increase of anti-histone antibodies [10–13] which react only with the most divergent parts of the *Leishmania* histones [12–14] resulting in absence of reactivity against mammalian histones [10]. Based on these findings, vaccine studies using DNA plasmids encoding nucleosomal histones [15,16] or nucleosomal histone-pulsed dendritic cells (DCs) [15,17] were conducted in mice suffering from cutaneous leishmaniasis (CL) or VL with significant protection.

DCs are potent professional antigen-presenting cells playing a central role in the induction of T cell immunity [18,19]. In addition, unmethylated CpG synthetic oligonucleotides (ODNs) have been used in many cases as adjuvant with a range of protein vaccines against infectious diseases, since they facilitate a Th1-mediated response by stimulating production of IL-12 and IFNγ [20]. Although, several vaccine strategies using antigen-pulsed bone marrow-derived DCs (BM-DCs), including nucleosomal histones in the presence of adjuvants have provided promising results [15,17,21–24], the use of *Leishmania* histone H1-pulsed DCs as a vaccine candidate against *Leishmania* has not been elucidated yet.

Taking the above into account, we investigated the protective potential of BM-DCs pulsed with *L. infantum* histone H1 (LeishH1)

Abbreviations: Ab, antibody; mAb, monoclonal antibody; FACS, fluorescence acquisition and cell sorting; SI, stimulation index.

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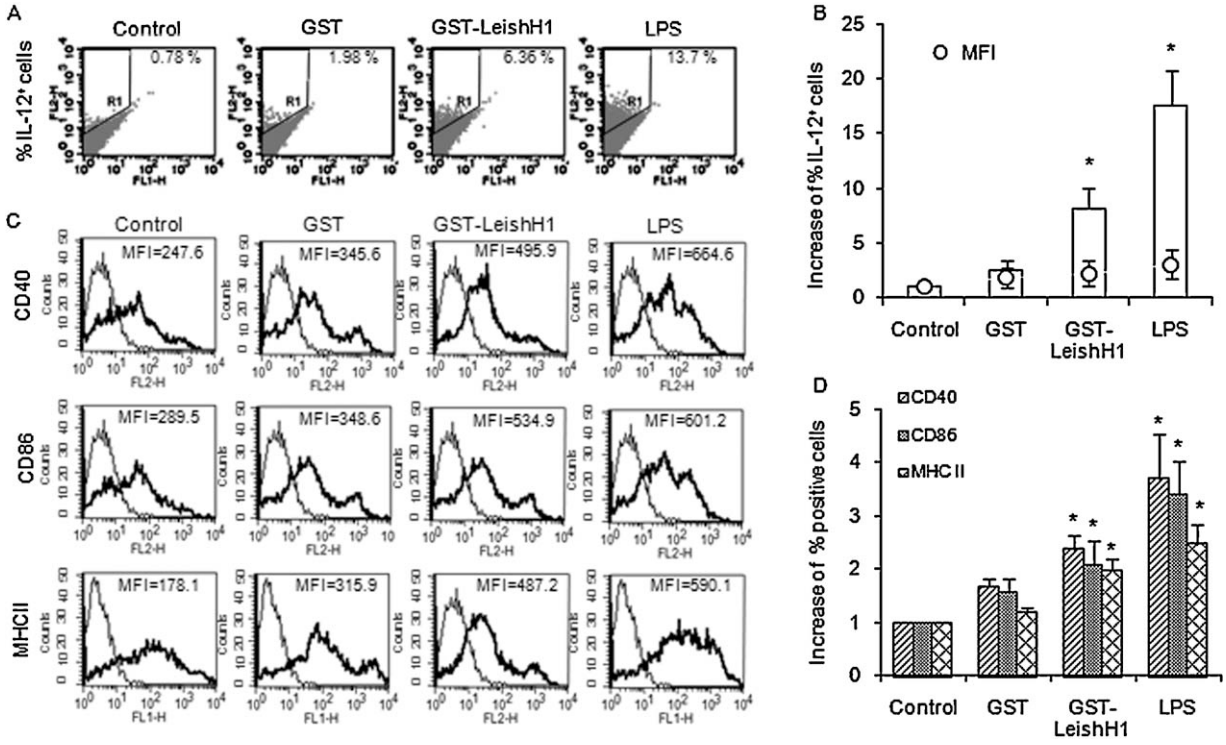

Fig. 1. Functional maturation of BM-DCs pulsed with (i) GST moiety (10 μg ml^{-1}) or (ii) GST-LeishH1 fusion protein (10 μg ml^{-1}) for 24 h. Unpulsed BM-DCs (control) and LPS-pulsed BM-DCs used as negative and positive control, respectively. (A) Dot plots for intracellular IL-12. (B) Percentage of IL-12$^+$ cells and their mean fluorescence intensity (MFI) were expressed as fold increase compared to unpulsed BM-DCs. (C) Expression of CD40, CD86 and MHCII surface markers is shown in the histograms (solid line). Fine line represent nonspecific fluorescence of cells stained with an isotype control Ab. (D) The diagram shows the fold increase of the percentage (%) of positive cells for CD40, CD86 and MHC class II surface markers and (C) and of their MFI. Dot plots and histograms depict one representative experiment; the results in the diagrams are the mean value of each cellular group from three independent experiments ± SD. Significant differences among groups are indicated by $^*P < 0.05$.

in a murine model of VL caused by *L. infantum* and explored the immune responses elicited after administration of those BM-DCs.

2. Materials and methods

2.1. Animals

Female BALB/c (H-2^d) mice, 6–8 weeks old, were used for our experimental purposes with prior approval by the Animal Bioethics Committee of the Hellenic Pasteur Institute (HPI; Athens, Hellas) regulating according to the EC Directive 1986/609 and the National Law 1992/2015. Mice were obtained from the breeding unit of the HPI and reared in institutional facilities under specific pathogen-free conditions, receiving a diet of commercial food pellets and water ad libitum.

2.2. Parasites

L. infantum (MON-1, MCAN/PT/98/IMT 244) was used in all experiments. Parasite virulence was maintained by monthly passage of 1×10^7 stationary phase promastigotes in BALB/c mice. Promastigotes were cultured in RPMI-1640 medium (Biochrom AG, Berlin, Germany) supplemented with 2 mM L-glutamine, 10 mM HEPES, 24 mM $NaHCO_3$, 0.05 mM β-mercaptoethanol, 100 U/ml penicillin, 100 μg ml^{-1} streptomycin and 10% (v/v) heat inactivated fetal bovine serum (FBS; Gibco, Paisley, UK) at 26 °C. For infection, BALB/c mice were injected intravenously via the lateral tail vein, with 1×10^7 second-passage promastigotes suspended in 100 μl PBS.

2.3. Preparation of soluble Leishmania antigen

Soluble *Leishmania* antigen was prepared from stationary phase promastigotes, as previously described [25].

2.4. Expression and purification of L. infantum histone 1

The LeishH1 gene DNA (GenBank accession No. AF469106) was amplified from genomic *L. infantum* DNA and cloned into the pGEX-4T1 plasmid (GE Healthcare, Buckinghamshire, UK) to generate an in frame GST-LeishH1 fusion protein as previously described [9].

GST or GST-LeishH1 were subsequently dialysed against sterile PBS and purified on a polymyxin B agarose column (Sigma, St. Louis, MO) to eliminate possible LPS contamination. Endotoxin concentrations were determined using a Chromogenic LAL kit (Bio-Whittaker, Walkersville, MD); GST and GST-LeishH1 proteins were essentially free of endotoxin (5 EU/mg). The final concentration and purity of protein was determined by SDS-PAGE.

2.5. CpG ODNs

A synthetic oligonucleotide (5-tccatgacgttcctgacgtt-3) that contains two unmethylated CpG motifs was purchased from Invivogen (ODN1826; San Diego, CA), resuspended according to manufacturer's instructions and stored at −20 °C until use.

2.6. BM-DCs generation

BM-DCs were generated in the presence of rmGM-CSF according to previous publication [24]. On day 8, nonadherent cells were collected and characterized; the percentage of CD11c$^+$CD8α$^-$ cells