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Original article

Lipooligosaccharide from *Bordetella pertussis* induces mature human monocyte-derived dendritic cells and drives a Th2 biased response

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Abstract

Bordetella pertussis has a distinctive cell wall lipooligosaccharide (LOS) that is released from the bacterium during bacterial division and killing. LOS directly participates in host-bacterial interactions, in particular influencing the dendritic cells' (DC) immune regulatory ability. We analyze LOS mediated toll-like receptor (TLR) activation and dissect the role played by LOS on human monocyte-derived (MD)DC functions and polarization of the host T cell response. LOS activates TLR4-dependent signaling and induces mature MDDC able to secrete IL-10. LOS-matured MDDC enhance allogeneic presentation and skew T helper (Th) cell polarization towards a Th2 phenotype. LOS protects MDDC from undergoing apoptosis, prolonging their longevity and their functions. Compared to *Escherichia coli* lipopolysaccharide (LPS), the classical DC maturation stimulus, LOS was a less efficient inducer of TLR4 signaling, MDDC maturation, IL-10 secretion and allogeneic T cell proliferation and it was not able to induce IL-12p70 production in MDDC. However, the MDDC apoptosis protection exerted by LOS and LPS were comparable. In conclusion, LOS treated MDDC are able to perform antigen presentation in a context that promotes licensing of Th2 effectors. Considering these properties, the use of LOS in the formulation of acellular pertussis vaccines to potentiate protective and adjuvant capacity should be taken into consideration.

Keywords: Dendritic cells; *Bordetella pertussis*; Lipooligosaccharide; T helper 2 response; Adjuvant

1. Introduction

Bordetella pertussis is the causative agent of whooping cough, a disease known to affect both adults and children with a high incidence of morbidity and mortality [1,2]. Pertussis is a multifactorial disease, the bacterium produces a range of toxins and adhesins, all of which are known to contribute to pathogenesis by interfering with the host immune response [1,3]. Immunity against *B. pertussis* develops after natural infection and confers a short-lived protection against subsequent infection. Immunization with whole cell pertussis vaccines (wP) protects against disease, but the reactogenicity of this vaccine has demanded the development of new generation acellular pertussis vaccines (aP) composed of purified putative protective antigens [1].

B. pertussis has a distinctive cell wall lipopolysaccharide (LPS) which is most accurately described as a lipooligosaccharide (LOS) [4,5] constituted by a penta-acylated lipid A domain and a core oligosaccharide, lacking O-side chain but in

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its place having a non-repeating trisaccharide [4]. LOS, although not secreted, can be released from the bacterium during bacterial division and killing and directly participates in host-bacterial interactions, thus it could be involved in the bacterial capacity to influence and modulate the host response.

A useful *ex vivo* model to evaluate the immunomodulatory properties of LOS is represented by human monocyte-derived dendritic cells (MDDC). DC are professional antigen presenting cells (APC) distributed in lymphoid and non-lymphoid organs [6]. They possess a wide array of receptors specialized in pathogen recognition which permit detection and identification of invading microbes [6]. An example is represented by the family of toll-like receptors (TLRs), which recognize microbial products representing danger signals for the host [7]. Upon activation DC undergo maturation and migrate to lymphoid organs where they encounter naïve T helper (Th) lymphocytes. Based on the expression of surface receptors and specific cytokines, mature DC drive Th1, Th2, Th17 or Treg immune responses [8]. The nature of these responses is largely determined by the type of microbial products encountered in the peripheral tissues during the immature phase [7].

We have previously shown that *B. pertussis* triggers the onset of the maturation program and modulates cytokine production of infected MDDC and although being unable to induce IL-12p70 (a crucial regulatory cytokine in the Th1 polarization of the immune response [9]), drives Th1 immunity [10,11], in accordance with the induction of Th1 response during the natural infections in infants and adults [12–15].

In the present study we dissect the role played by LOS from *B. pertussis* on human MDDC functions and the consequent polarization of T cell response, comparing the modulatory properties of LOS with *Escherichia coli* (Ec) LPS, chosen as a prototypic TLR4 ligand and DC maturation stimulus.

2. Materials and methods

2.1. Reagents

Ec LPS phorbol myristate acetate (PMA), ionomycin and brefeldin A were from Sigma Chemicals (St. Louis, MO). Purified *E. coli* LPS and synthetic bacterial lipoprotein S-[2,3-bis(palmitoloxy)-(2RS)-propyl]-[R]-[cysteinyl-[S]-lysil-[S]-lysine × 3 CF3COOH (Pam2CSK4) were purchased from InvivoGen Europe (Toulouse, France).

Human recombinant (r) granulocyte-macrophage colony-stimulating factor (GM-CSF) and rIL-4 were from Novartis Pharma (Basel, Switzerland). Culture supernatants (4%, v/v) of IL-4-62 cell line were also used as source of IL-4 [16]. rIL-2 was obtained from Roche (Basel, Switzerland).

2.2. B. pertussis *LOS purification*

LOS from *B. pertussis* was purified and characterized as described in ref. [5]. Briefly, BP338 *B. pertussis* LOS was purified by incubating bacteria with proteinase K (Sigma), followed by acetone precipitation. SDS-PAGE and silver staining of the purified LOS revealed two distinct bands, referred to as bands A and B, and their identity was verified by immunoblot [5].

2.3. Cell lines

Human epithelial kidney (HEK) 293 cells stably transfected with human TLR4, MD2 and CD14 (HEK/TLR4) were purchased from InvivoGen; HEK 293 cells stably transfected with human TLR2 (HEK/TLR2) were kindly provided by Dr. Kate A. Fitzgerald [17]. The HEK/TLR clones were grown in standard Dulbecco's modified Eagle's medium (DMEM) with heat-inactivated 10% LPS-screened fetal calf serum (FCS) (LAL <1 ng/ml) (Hyclone Laboratories, Logan, UT) supplemented with 1 mM sodium pyruvate, 0.1 mM non-essential amino acids, 2 mM L-glutamine, all from Hyclone Laboratories and normocin™ (100 μg/ml, InvivoGen) (hereafter defined HEK medium), in a 5% saturated CO_2 atmosphere at 37 °C. Blasticidin (0.2 mM, InvivoGen) and HygroGold™ (50 μg/ml, InvivoGen) were added to HEK/TLR4 cultures, while G418 Sulfate (0.6 mM, InvivoGen) was added to HEK/TLR2 cultures.

2.4. Stable transfection with NF-κB-inducible reporter plasmid pNifty2-SEAP

HEK/TLR cells were seeded into 6-well plates at a density of 4×10^5 cells in 0.5 ml HEK medium per well and transfected 24 h later with 100 μl of Lyovec™ (InvivoGen) mixed with 5 μg of p-Nifty2-SEAP (InvivoGen). A total of 24 h after transfection, cells were selected with Zeocin™ (InvivoGen). Stably transfected HEK/TLR/p-Nifty2-SEAP clones were obtained after two weeks.

2.5. TLR signaling assay

The induction of TLR signaling in HEK/TLR/p-Nifty2-SEAP clones was assessed by measuring secreted alkaline phosphatase (SEAP) activity using QUANTI-Blue™ colorimetric assay (InvivoGen). Briefly, cells were seeded for 48 h into 24-well plates at a density of 2×10^5 cells in 0.5 ml HEK medium added with specific selection antibiotics, cells were then either untreated (none) or treated for 16 h at the indicated doses with *B. pertussis* LOS, ultrapure Ec LPS or Pam2CSK4. Supernatants (10 μl) were then transferred to a 96-well plate and incubated at 37 °C with QUANTI-Blue™ (200 μl). SEAP activity was measured by reading optical density at 655 nm with a 3550-UV Microplate Reader (BioRad, Philadelphia, PA). Data are reported as the fold induction of SEAP activity over untreated controls.

2.6. Purification and culture of MDDC

Monocytes were purified by positive sorting using CD14-mAb-conjugated magnetic microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany) from peripheral blood mononuclear cells obtained after Ficoll gradient (Lympholyte-H, Cedarlane, Hornby, Ontario) [16]. CD14 cells were cultured