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Original article

Chlamydia pneumoniae modulates human monocyte-derived dendritic cells functions driving the induction of a Type 1/Type 17 inflammatory response

Davide Flego[1,2], Manuela Bianco[2], Adriano Quattrini, Fabiola Mancini, Maria Carollo, Ilaria Schiavoni, Alessandra Ciervo, Clara M. Ausiello, Giorgio Fedele*

Department of Infectious, Parasitic and Immune-Mediated Diseases, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy

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Abstract

Chlamydia pneumoniae is a respiratory pathogen involved in the onset of chronic inflammatory pathologies. Dendritic cells (DC), are major players in spreading of *C. pneumoniae* from the lungs, a crucial step leading to disseminated infections. Less is known concerning modulation of DC functions consequent to encounter with the bacterium. In order to address this aspect, human monocyte-derived (MD)DC were infected with *C. pneumoniae*. After internalization bacterial counts increased in MDDC, as well as the expression of *CPn1046*, a gene involved in bacterial metabolism, with a peak 48 h after the infection. Infected MDDC switched to the mature stage, produced IL-12p70, IL-1β, IL-6, and IL-10, and drove a mixed Type 1/Type 17 polarization. Intracellular pathways triggered by *C. pneumoniae* involved Toll-like receptor (TLR) 2. Indeed, TLR2 was activated by *C. pneumoniae* in transfected HEK 293 cells, and *C. pneumoniae*-mediated phosphorylation of ERK1/2 was inhibited by an anti-TLR2 antibody in MDDC. When an ERK1/2 inhibitor was used, IL-12p70 and IL-10 release by MDDC was reduced and T cell polarization shifted towards a Type 2 profile. Overall, our findings unveiled the role played by TLR2 and ERK1/2 induced by *C. pneumoniae* to affect DC functions in a way that contributes to a Type 1/Type 17 pro-inflammatory response.

Keywords: *Chlamydia pneumoniae*; Dendritic cells; Cytokines; T-helper cells; Toll-like receptor

1. Introduction

Chlamydia pneumoniae is an obligate intracellular bacterium causing respiratory infections such as acute pneumonia, bronchitis, sinusitis and pharyngitis [1]. Up to 10% of cases of community-acquired pneumonia are apparently caused by *C. pneumoniae* and re-infections are common, with studies indicating seroprevalence in 80% of adults [2].

Persistent *C. pneumoniae* infections have been generally associated with bacterial entry into an alternative developmental cycle, which involves conversion to a persistent state secluded from the cytoplasmic environment [1]. There is an increasing evidence for an association between persistent *C. pneumoniae* infections and a range of chronic diseases including atherosclerosis, asthma, arthritis, multiple sclerosis and Alzheimer's disease [3–7].

Although the mechanisms by which persistent *C. pneumoniae* may contribute to progression of chronic diseases are still elusive, it has been shown that the bacterium is able to lead to enhanced expression of inflammatory mediators in a large variety of cells [8–11].

Dendritic cells (DC) are key players of the immune response that might be involved in *C. pneumoniae*-induced chronic disease state. Indeed, it is well known that dysregulated DC functions may lead to the development of inflammatory diseases [12]. Remarkably, DC have been shown to internalize *C. pneumoniae in vitro* [13–15] and it has been

* Corresponding author. Tel.: +39 06 4990 2890; fax: +39 06 4990 3168.
E-mail addresses: fedele@iss.it, giorgio.fedele@iss.it (G. Fedele).
[1] Present address: Istituto di Cardiologia, Catholic University of Sacred Heart, Rome, Italy.
[2] DF and MB equally contributed to this study.

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demonstrated that *C. pneumoniae* is present in the cytoplasm of DC in atherosclerotic lesions [16].

The aim of this study was to evaluate how *C. pneumoniae* could influence the functions of human DC and understand whether infection could favor the onset of a pro-inflammatory environment. Internalization and metabolic activity of bacteria in monocyte-derived dendritic cell (MDDC) was assessed as well as the activation of key MDDC functions, including resistance to apoptosis, phenotypic maturation, cytokine production and polarization of T lymphocytes. The role of Toll-like receptor (TLR) 2 and TLR4 and the induction of mitogen-activated protein kinases (MAPK) pathways were also investigated.

2. Material and methods

2.1. Ethics statement

This study was conducted according to the principles expressed in the Declaration of Helsinki. All blood donors provided written informed consent for the collection of samples and subsequent analysis, and the blood samples were processed anonymously.

2.2. Propagation of C. pneumoniae

C. pneumoniae strain Parola was propagated in Hep-2 cell monolayers (American Type Culture Collection, Rockville MD), at a multiplicity of infection of 10^2 infective units per cell. Infection was achieved by centrifugation at 800× *g* for 1 hour (h) at 37 °C in 6-well plates (Costar, Corning Life Sciences), and infected cultures were incubated for 72 h at 37 °C under 5% CO_2. *C. pneumoniae* was harvested by disruption of monolayers with sterile glass beads, and after a low-speed centrifugation at 200× *g*, elementary bodies were purified by Renografin gradient (Patheon Italia). Aliquots of purified bacteria were titrated by Real-time PCR [17] and stored at −70 °C in sucrose–phosphate–glutamate acid until used.

2.3. Purification and culture of MDDC

Human monocytes were purified from peripheral blood of healthy blood donors (courtesy of Dr. Girelli, "Centro Trasfusionale Policlinico Umberto I," University La Sapienza, Rome, Italy) after Ficoll gradient (lympholyte-H; Cedarlane). $CD14^+$ cells were further purified by positive sorting through CD14 mAb-conjugated magnetic microbeads (Miltenyi Biotec) and cultured in 75 cm^2 flasks (Costar, Corning Life Sciences) in RPMI 1640 medium (Life Technologies Invitrogen), supplemented with heat-inactivated 10% LPS-screened FCS, 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, 2 mM L-glutamine, 25 mM HEPES, 100 U/ml penicillin, 100 μg/ml streptomycin (all from EuroClone), and 0.05 mM 2-βmercaptoethanol (Sigma Chemicals) in the presence of human recombinant GM-CSF (25 ng/ml; R&D Systems) and IL-4 (25 ng/ml; R&D Systems). After 6 days, immature MDDC were washed with RPMI 1640 medium and analyzed by cytofluorometry (FACScan, BD Biosciences) for the expression of surface markers. MDDCs were used in the experiments if >85% CD1a and <5% CD14.

2.4. MDDC infection and C. pneumoniae internalization

MDDCs (10^6 cell/ml) were resuspended in complete medium without antibiotics and incubated with bacteria for 2 h with a multiplicity of infection (MOI) of 1 and 4. MDDC were then washed and resuspended in complete medium. Internalized bacteria were enumerated at 24 h, 48 h and 72 h time-points by quantitative Real-time PCR of the chlamydial *Pst I* gene as previously described [17].

2.5. C. pneumoniae activity by bacterial CPn1046 quantitative transcript assay

The assessment of bacterial activity was performed by quantitative reverse transcription real-time PCR through the measurement of *CPn1046* RNA transcripts during the time points of infection.

Total RNA was extracted from infected cells using an RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions with the protocol for bacterial cells. The concentration of RNA was quantified by spectrophotometry, and RNA was stored at −70 °C until used. The extracted RNAs were treated with DNase (DNA-free; Ambion) to eliminate the contaminating DNA.

One-step quantitative reverse transcription real-time PCR was conducted using QuantiTect SYBR green RT-PCR (Qiagen). To check the possible DNA contamination in each RNA sample, a real-time PCR was also performed in parallel using the QuantiTect SYBR green PCR (Qiagen) and same primer sets. An aliquot (100 ng) of the RNA preparation samples was used and the relative reaction mixtures and the amplification procedures were carried out according to the manufacturer's guidelines for the LightCycler instrument. All reactions were performed in four independent PCR runs. Two different primer pair sets previously described were used [18] and were:

16S rRNA-F (5′-GGATTTATTGGGCGTAAAGG-3′)
16S rRNA-R (5′-TCCACATCAAGTATGCATCG-3′)
CPn1046-F (5′-TGGAGTTGATGTATTGAAGG-3′)
CPn 1046-R (5′-TCTTATCAGTTCTCCTCAGG-3′)

The PCR conditions consisted of one cycle at 50 °C for 30 min; one cycle at 95 °C for 15 min; 40 cycles at 95 °C for 5 s, 56 °C for 15 s, and 72 °C for 15 s with the ramp of 20 °C/s for each cycle. The change in fluorescence of SYBR green dye in every cycle was monitored by the system software, melting curve analysis from 70 to 90 °C was acquired, and the threshold cycle (CT) above the background for each reaction was calculated.

The mean *CPn1046* RNA copy number value obtained for each sample was divided by the corresponding mean 16S rRNA value to standardize for the number of chlamydial bodies present in each preparation.