Microbial Pathogenesis 45 (2008) 290–296

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

Inhibition of lymphocyte CD3 expression by *Chlamydophila pneumoniae* infection

Hiroyuki Yamaguchi [a,b,*], Junji Matsuo [b], Shigehiro Sugimoto [a], Maki Utsumi [a], Yoshimasa Yamamoto [a]

[a] Laboratory of Molecular Microbiology, Department of Bioinformatics, Graduate School of Medicine, Osaka University, 1-7 Yamadaoka, Suita, Osaka 565-0871, Japan
[b] Department of Medical Laboratory Sciences, Graduate School of Health Sciences, Hokkaido University, Nishi-5 Kita-12 Jo, Kita-ku, Sapporo, Hokkaido 060-0812, Japan

ARTICLE INFO

Article history:
Received 10 January 2008
Received in revised form 16 June 2008
Accepted 26 June 2008
Available online 12 July 2008

Keywords:
Chlamydophila pneumoniae
lymphocytes
Molt-4 cells
CD3
prostaglandin E_2

ABSTRACT

Since lymphocytes are a major immune cell besides macrophages in the development of atherosclerosis, interaction between lymphocytes and *Chlamydophila pneumoniae* may contribute to the pathogenesis of chronic inflammatory diseases associated with *C. pneumoniae*. In this regard, we examined a possible alteration of CD3 expression of human lymphocyte Molt-4 cells by *C. pneumoniae* infection. The expression levels of CD3 molecules of lymphocyte Molt-4 cells were significantly decreased by *C. pneumoniae* infection. In contrast, heat-killed *C. pneumoniae* as well as mock (cell lysates) did not cause any alteration of CD3 expression of the cells. Treatment of the infected cells with NS-398 (cyclo-oxyganase-2 inhibitor) or AH-23848 (EP_4 prostanoid receptor antagonist) abolished the inhibition of CD3 expression. The enhanced prostaglandin E_2 (PGE_2) productions in the culture supernatants of infected cells were confirmed by competitive enzyme-immunosorbent assay (ELISA). *C. pneumoniae* infection of enriched lymphocytes from human peripheral blood mononuclear cells also induced a decrease of CD3 expression. Thus, *C. pneumoniae* infection of lymphocytes induces a decrease of CD3 expression mediated by possibly PGE_2 production.

1. Introduction

Chlamydophila pneumoniae is an obligate intracellular bacterium associated with human respiratory pathogen causing pneumonia [1–3]. Current studies indicate that this organism may be implicated in the induction of chronic inflammatory diseases such as asthma, arthritis, endocarditis, and atherosclerosis [4–7]. However, although accumulating studies indicated a possible linking *C. pneumoniae* infection and chronic inflammatory diseases, the mechanisms for development of such disease by this organism are not clear [8]. Lymphocytes are a major immune cell besides macrophages in the development of chronic inflammatory diseases, such as atherosclerosis [9, 10]. Therefore, a direct interaction between lymphocytes and this pathogen, if it occurs, may contribute to the pathogenesis of chronic inflammatory diseases associated with *C. pneumoniae* infection. Kaul et al. reported that *C. pneumoniae* DNA could be recovered from $CD3^+$ peripheral blood leukocytes obtained from patients attending a cardiology [11]. In addition, our studies also demonstrated that *C. pneumoniae* infects and multiplies in lymphocytes in vitro [12, 13]. These findings indicate that lymphocytes are a potential host cell for *C. pneumoniae* infection. Therefore, it can be possible that immune functions of lymphocytes infected with *C. pneumoniae* may be altered and such alterations lead an inappropriate immune response to stimulation, which eventually contribute to the pathogenesis of the disease.

CD3 receptor is a crucial molecule in T cell signal transduction [14–16]. Although its expression of cell is constitutive, dynamic regulation of T-cell receptor (TCR)/CD3 complex expression level is probably the most important mechanism allowing T cells to calibrate their response to different levels of stimuli or to sense harmful pathogen in cells. Therefore, a possible alteration of CD3 expression of lymphocytes by *C. pneumoniae* infection, if it occurs, may be a critical in the pathogenesis of the diseases associated with this pathogen. In this regard, in the present study, we examined a possible alteration of CD3 expression of lymphocytes by *C. pneumoniae* infection.

2. Results

2.1. C. pneumoniae *infection resulted in a decrease of CD3 expression of Molt-4 cells*

Fig. 1 shows representative micrographs and dot plots of the cells with or without infection at 72 h after incubation. Flow data of double-labeled cells indicated a decrease of CD3 expression of

* Corresponding author. Department of Medical Laboratory Sciences, Graduate School of Health Sciences, Hokkaido University, Nishi-5 Kita-12 Jo, Kita-ku, Sapporo, Hokkaido 060-0812, Japan. Tel./fax: +81 11 706 3326.
E-mail address: hiroyuki@med.hokudai.ac.jp (H. Yamaguchi).

0882-4010/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.micpath.2008.06.005

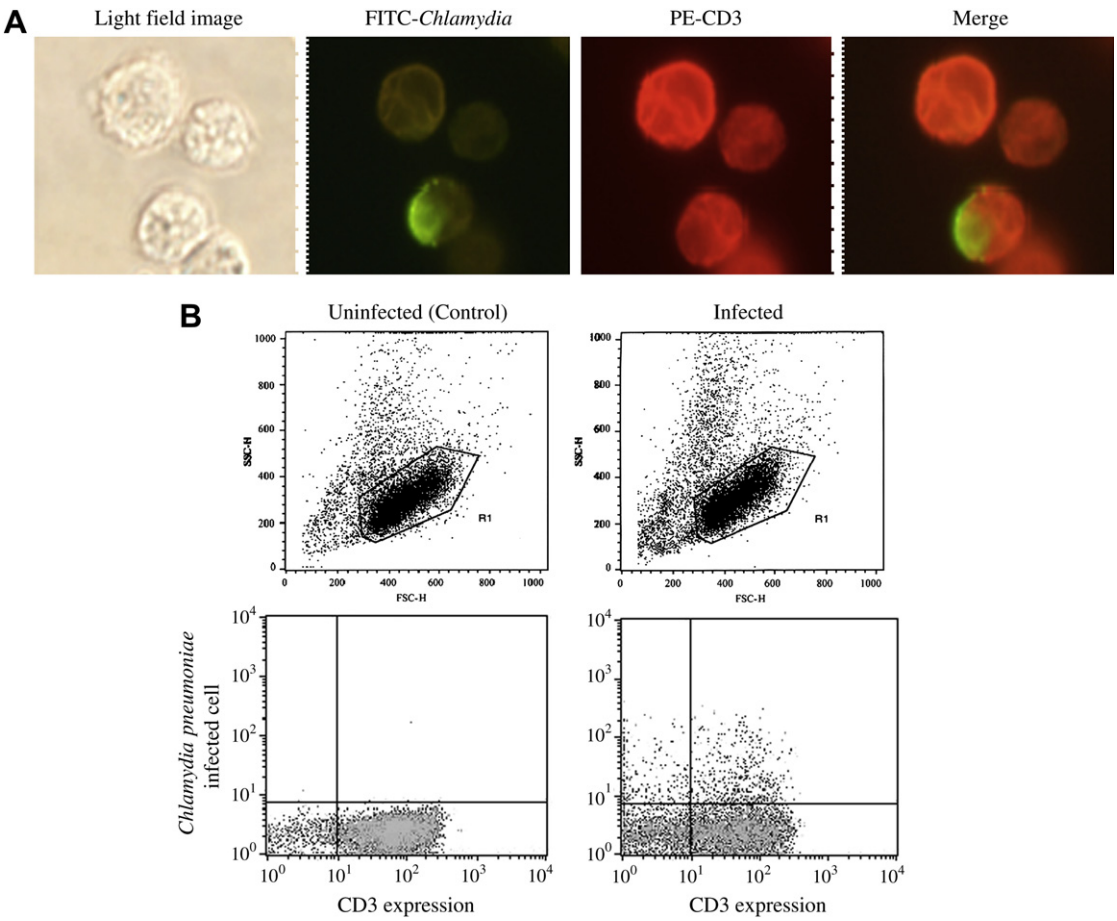

Fig. 1. Representative double-fluorescence staining micrographs (A) and dot-plot images (B) of *C. pneumoniae*-infected lymphocyte Molt-4 cells at 72 h after incubation. The cells were infected with bacteria at MOI of 10. The cells treated with the permeabilization kit (see Section 5) were stained with PE-conjugated anti-CD3 MoAb and FITC-conjugated anti-*Chlamydia*-MoAb. The specimens were then analyzed by fluorescence microscopy and flow cytometry. (A) Red and green colors are indicating CD3 expression and *C. pneumoniae* inclusion of lymphocyte Molt-4 cells, respectively. Magnification, ×1000. (B) Gated cells (R1) are showing analyzed cell population excluding cell debris and dead cells (upper panels). The dot-plot intensity showing CD3 expression of infected cells was lower than that of uninfected cells (lower panels). The mean intensities of CD3 expression of uninfected and infected cells were 75.06 and 51.46, respectively. In this case, the reduction rate showing a decrease of CD3 expression of infected cells compared with that of uninfected cells was 23.6%. The infectious rate in culture was approximately 10%.

infected cells compared with CD3 expression of uninfected cells. There was no difference between dot-plot patterns with side scatter (SSC) and forward scatter (FSC), indicating the influence of cell debris and dead cells causing artificial staining results was minimal. The micrographs also showed that the double-labeled cells were morphologically normal and this staining method was certainly dyed in different colors. Kinetic change of CD3 expression of Molt-4 cells by *C. pneumoniae* infection was assessed as follows. The cells were infected at MOI of 1 or 10 and incubated for up to 72 h. At various time points, CD3 expression was measured by flow cytometry using anti-CD3 MoAb. As shown in Fig. 2, *C. pneumoniae* infection induced a decrease of CD3 expression depending on MOI. The decreasing rates of CD3 molecules of Molt-4 cells became the maximum at 72 h after infection. In contrast, α 4 β 1 integrin very late activation antigen-4 (VLA-4) expression was not altered by the infection for up to 72 h. Heat-killed bacteria and HEp-2 cell lysates (mock) did not cause any alteration of CD3 expression of Molt-4 cells (Fig. 3).

2.2. Inhibition of CD3 expression of human peripheral blood lymphocytes by C. pneumoniae infection

In order to determine a possible alteration of CD3 expression of primary lymphocytes by infection as observed in established Molt-4 cells, human lymphocytes enriched from peripheral blood mononuclear cells (PBMCs) were infected with or without viable bacteria in the presence or absence of cycloheximide for up to 48 h, and alteration of CD3 expression of primary lymphocytes were assessed at 24 and 48 h after infection. As shown in Fig. 4, the infected cells showed an obvious decrease of CD3 expression of primary lymphocytes at 48 h after infection. The inhibition of CD3 expression was completely diminished by the treatment with cycloheximide, which inhibits host cell protein synthesis.

2.3. Inhibition of CD3 expression mediated by soluble factors

Alteration of CD3 expression of lymphocytes may be induced by several mechanisms, including an involvement of soluble factors produced by lymphocytes. In order to determine such a possibility in the inhibition induced by *C. pneumoniae* infection, transwell plates with a membrane were utilized for assessment of possible involvement of soluble factors. Both chambers of the transwell plates were cultured with Molt-4 cells. The cells in the upper chamber were infected with *C. pneumoniae* at MOI of 1–50, but the lower chamber contained only cells without infection. The percentage inhibition of CD3 expression of both chambers determined by flow cytometry was measured at 72 h after incubation. As shown in Fig. 5, the infected Molt-4 cells in the upper chamber