Veterinary Immunology and Immunopathology 136 (2010) 116–121

Contents lists available at ScienceDirect

Veterinary Immunology and Immunopathology

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

Short communication

# Global suppression of mitogen-activated ovine peripheral blood mononuclear cells by surface protein activity from *Mycoplasma ovipneumoniae*

W. Shahzad [a,b], Adebowale Titilayo Phillip Ajuwape [a,c], Ricardo Francisco Rosenbusch [a,*]

[a] Department of Veterinary Microbiology and Preventive Medicine, College of Veterinary Medicine, Iowa State University, VMRI-5, 1802 University Boulevard, Ames, IA 50011, USA
[b] Department of Clinical Medicine, University of Veterinary & Animal Sciences, Lahore, Pakistan
[c] Department of Veterinary Microbiology and Parasitology, University of Ibadan, Ibadan, Nigeria

ARTICLE INFO

*Article history:*
Received 25 October 2009
Received in revised form 26 December 2009
Accepted 1 February 2010

*Keywords:*
*Mycoplasma ovipneumoniae*
Lymphocyte subsets
Lymphocyte suppression
Sheep and goat pneumonia

ABSTRACT

*Mycoplasma ovipneumoniae* is associated with chronic non-progressive pneumonia of sheep and goats. As with many other mycoplasmas involved in animal diseases, protective immune responses have not been achieved with vaccines, even though antibody responses can be obtained. This study focuses on characterizing the interaction of *M. ovipneumoniae* with ovine PBMC using carboxy-fluorescein-succinimidyl-ester (CFSE) loading and flow cytometry to measure lymphoid cell division. *M. ovipneumoniae* induced a strong *in vitro* polyclonal suppression of $CD4^+$, $CD8^+$, and B blood lymphocyte subsets. The suppressive activity could be destroyed by heating to 60 °C, and partially impaired by formalin and binary ethyleneimine treatment that abolished its viability. The activity resided on the surface-exposed membrane protein fraction of the mycoplasma, since mild trypsin treatment not affecting viability was shown to reduce suppressive activity. Trypsin-treated mycoplasma regained suppressive activity once the mycoplasma was allowed to re-synthesize its surface proteins. Implications for the design of vaccines against *M. ovipneumoniae* are discussed.

© 2010 Elsevier B.V. All rights reserved.

## 1. Introduction

Acquired immune protection against mycoplasmal diseases of the respiratory tract of man and animals has been difficult to achieve. Antibody and CMI responses have been considered necessary, although these host reactions have been implicated in both protection and lesion formation (Jones and Simecka, 2003). In particular, the protective role of CMI responses has been shown to vary among mycoplasma species; a protective role was described for *Mycoplasma pulmonis* (Denny et al., 1972), while inconsistent association of CMI with protection was reported for *Mycoplasma hyopneumoniae* (Messier et al., 1990; Hoelzle et al., 2009). Studies of the *in vitro* interaction of mycoplasmas with lymphocytes have been carried out to understand specific components of the role of CMI. An arginine deiminase activity released from *Mycoplasma arginini* was shown to suppress B cell activation by mitogens without affecting T cells (Foresman et al., 1989). This activity has also been shown to be expressed by multiple Gram positive species including *Streptococcus pyogenes* (Degnan et al., 1998). In contrast, heat-labile surface factors in *Mycoplasma mycoides* subsp. *mycoides* small colony type suppressed T cells predominantly, and the suppressive effect on B cells was considered to be mediated by the T cell effect (Dedieu and Balcer-Rodrigues, 2006). Global suppression of

Abbreviations: BEI, binary ethyleneimine; CFSE, carboxy-fluorescein-succinimidyl-ester; CMI, cell-mediated immune response; PI, propidium iodide.

* Corresponding author. Tel.: +1 515 294 6170; fax: +1 515 294 1401.
*E-mail address:* rfrosenb@iastate.edu (R.F. Rosenbusch).

0165-2427/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetimm.2010.02.001

peripheral lymphocytes was observed to be caused by the surface-exposed N-terminal peptide of a membrane protein of *Mycoplasma bovis* (Vanden Bush and Rosenbusch, 2004). Hydrogen peroxide release by some mycoplasmas has been implicated in epithelial cell death (Bishof et al., 2008), although it has only been associated with activation rather than suppression of lymphocytes (Arai et al., 1983).

*Mycoplasma ovipneumoniae* is an emerging veterinary pathogen (Parham et al., 2006; Besser et al., 2008) isolated from the respiratory tract of sheep and goats as well as wild small ruminants, and is associated with chronic non-progressive pneumonia and economic losses (Sullivan et al., 1973; Alley et al., 1975). Attempts to develop an inactivated bacterin for this infection have resulted in no measurable protective effect (Rosenbusch, pers. comm.). It is known that antibody responses appear slowly after the infection, which might be partly due to immune suppression or failure of the immune system to generate protective immunity against the organism (Mohan et al., 1992; Niang et al., 1998a, 1999). Reasons for the failure are not known, but marked variation in the *M. ovipneumoniae* organisms isolated from sheep and expression of a polysaccharide capsule by the organisms are recognized (Niang et al., 1998b, 1999). Earlier investigations showed that the bronchus-associated lymphoid tissue in infected animals had enlarged germinal centres composed primarily of IgG-producing B lymphocytes, suggesting that mature activated T lymphocytes, particularly the $CD4^+$ subset, and IgG B cells played a major role in the immune response (Niang et al., 1998a). The current investigation was designed to find out if *M. ovipneumoniae* will suppress all or some of the activated ovine lymphocyte subsets (CD4, CD8 and B) *in vitro* and to characterize the *M. ovipneumoniae* components that effect lymphocyte suppression.

## 2. Materials and methods

### 2.1. Mycoplasma Ag preparation

Strain 56 of *M. ovipneumoniae* was recovered from Iowa lambs with chronic cough (Harvey et al., 2007). The mycoplasma was grown in modified Friis broth at 37 °C for 24 h as previously described (Knudtson et al., 1986). Growth was harvested by centrifugation (18,000 × *g* for 20 min at 4 °C), washed three times in PBS pH 7.4, and stored as Ag at −70 °C at a concentration of 1 mg/ml (by Bio-Rad DC protein assay, Bio-Rad, Hercules, CA, USA) in RPMI-1640 complete medium (RPMI-supplemented with 100 μg/ml glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, 5 μM β-mercaptoethanol, 25 mM HEPES, 1 mM sodium pyruvate and 10% FBS, all from Sigma–Aldrich, St. Louis, MO) and used at concentrations ranging from 3.1 to 200 ng/well.

### 2.2. Inactivation of mycoplasma

Heat inactivation of *M. ovipneumoniae* Ag was performed for 15, 30, 60 and 120 min at 60 °C as described (De la Fe et al., 2004). Inactivation of *M. ovipneumoniae* antigen with formalin was as described by De la Fe et al. (2004) with modifications. Briefly, ultra pure formaldehyde (Polysciences, Warrington, PA) to a final concentration of 0.2% was added to Ag followed by NaOH to 23 mM, and incubation at 8 °C for 18 h. Excess formaldehyde was reduced by addition of sodium bisulfite (at 23 mM final concentration) and incubation at 37 °C for 120 min. The procedure used for inactivation with binary ethyleneimine (BEI), was modified from that described by De la Fe et al. (2004). Washed mycoplasma cells were resuspended to 1 mg/ml protein in prewarmed inactivation buffer (Hank's Balanced Salt Solution, 1% bovine serum albumin, 5 mM EDTA, pH 7.4). A stock of 0.1 M BEI was prepared by dissolving 2-bromo-ethylamine (Sigma) in 0.175N NaOH. This was added at 3% (v/v) to the mycoplasma suspension and incubated at 37 °C for 24 h with rotation. Then 1 M sodium thiosulfate was added at 10% of the original volume of 0.1 M BEI and incubated at 37 °C for 2 h with rotation. In all cases, inactivation of *M. ovipneumoniae* was confirmed by plating a 10 μl aliquot on modified Friis agar, with absence of colonial growth after 10 days of incubation. Although no attempt was made to eliminate residual inactivation chemicals, all antigen preparations were diluted at least 500-fold as used in assays, assuring that residues would not affect the blastogenesis assay.

### 2.3. Trypsin treatments of mycoplasma cells

Washed mycoplasma cells resuspended in PBS at 1 mg/ml were subjected to trypsin treatment. Twenty μl of this Ag was centrifuged at 14,000 rpm for 10 min at 4 °C, resuspended in 400 μl of trypsin diluent (Dulbecco's PBS without $Ca^{2+}$ or $Mg^{2+}$, pH 8.0, supplemented with 0.1 mM $CaCl_2$). This preparation was aliquoted into 4 tubes of 100 μl each, centrifuged at 14,000 rpm at 4 °C for 10 min, and two of them resuspended in 100 μl of 500 μg/ml trypsin (trypsin 1:200, Gibco, Grand Island, NY) in trypsin diluent. The other two were resuspended in 100 μl of 100 μg/ml trypsin in trypsin diluent. The reactions were incubated at 37 °C for 60 min with rotation and then centrifuged at 14,000 rpm for 10 min at 4 °C. Centrifuged mycoplasmas from one of the 100 μg/ml and one of the 500 μg/ml trypsinization tubes were resuspended in 40 μl RPMI-complete and stored immediately on ice. The other two tubes left over were each supplemented with 1 ml of warm modified Friis broth and incubated at 37 °C for 60 min to allow re-synthesis of surface proteins. The tubes were centrifuged at 14,000 rpm for 10 min at 4 °C, and each then resuspended in 40 μl RPMI-complete. All four trypsin-treated mycoplasma preparations were then used immediately at 200 ng/well for lymphocyte blastogenesis.

### 2.4. Blastogenesis assay

Five-month-old lambs (Suffolk breed) free of respiratory symptoms were secured as blood donors. The lambs were tested for *M. ovipneumoniae* infection by culturing nasal swabs in modified Friis broth and agar. Approved Institutional Animal Care guidelines were followed for work with these animals. Blood collection (30 ml) was done from the jugular vein into Alsever's solution. Blood samples were diluted 1:1 with 37 °C PBS and layered on Histopaque-1077 (Sigma). Upon centrifugation (500 × *g*

Download English Version:

https://daneshyari.com/en/article/2462399

Download Persian Version:

https://daneshyari.com/article/2462399

Daneshyari.com