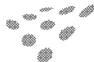
Available online at www.sciencedirect.com



ScienceDirect

Agricultural Sciences in China
2007, 6(10): 1262-1268

October 2007

Recombinant Galectins of *Hemonchus contortus* Inhibit Goat Cytokine mRNA Transcription of Peripheral Blood Mononuclear Cells *in vitro*

SUN Yan-ming, YAN Ruo-feng, XU Li-xin, Charles I Muleke and LI Xiang-rui

College of Veterinary Medicine, Nanjing Agricultural University, Nanjing 210095, P.R.China

Abstract

The effects of recombinant galectins of male and female *Hemonchus contortus* (*Hco*-gal-m/f) on the mRNA levels of IL-1β, IL-2, IL-4, IL-6, interferon-γ (IFN-γ), and tumor necrosis factor-α (TNF-α) of goat peripheral blood mononuclear cells (PBMCs) were examined in this study. Blood samples were collected from five randomly selected two-year-old healthy goats. PBMCs were separated and cultured *in vitro* with varying concentration galectins (*Hco*-gal-m/f) of 0, 10, 20, and 40 μg mL^{-1}. Semi-quantitative reverse transcription RT-PCR was employed to test the synthesis of mRNA. The results showed that the mRNA transcriptions of IL-1β, IL-2, IL-4, IL-6, IFN-γ, and TNF-α in the PBMCs were inhibited by *Hco*-gal-m in a dose-dependent manner. *Hco*-gal-f inhibited mRNA synthesis of IL-1β, IL-4, IFN-γ and TNF-α of the same cells in similar fashion. The findings suggested that the recombinant galectin proteins of *H. contortus* could decrease the transcription of cytokines *in vitro*.

Key words: *Hemonchus contortus*, recombinant galectin, cytokines, goat

INTRODUCTION

Galectins represent a family of lectins which occur in vertebrates and invertebrates, and specifically bind to β-galactoside sugars in a metal-independent manner, as conferred by conserved amino acid residues present within a carbohydrate recognition domain (CRD) (Hirabayashi *et al.* 1992; Barondes *et al.* 1994; Kasai and Hirabayashi 1996). To date, 14 mammalian galectins have been identified in a variety of animal tissues (Rabinovich *et al.* 2002). Galectins have been isolated from a number of helminth parasites including *Onchocerca volvulus* (Klion and Donelson 1994), *Ladorsagia circumcincta* (Newton *et al.* 1997), *Haemonchus contortus*, and *Trichostrongylus colubriformis* (Greenhalgh *et al.* 1999).

Recent evidence suggests that galectins act as master regulators of immune cell homeostasis (Rabriel *et al.* 2002). Cell galectin-3 is an amplifier of inflammatory cascade, while galectin-1 triggers homeostatic signals to block the T effector functions. These carbohydrate-binding proteins participate in the homeostasis of the inflammatory response, either by regulating cell survival and signaling, influencing cell growth and chemotaxis, interfering with cytokine secretion, mediating cell-cell and cell-matrix interactions or by influencing tumor progression and metastasis (Rossiter *et al.* 1997; Cooper and Barondes 1999).

Infections with *H. contortus*, a voracious blood-feeding nematode, causes a major drawback to cattle, sheep, and goat production in many parts of the world. Adult worms cause severe anaemia, poor growth rate, weight loss and death, especially in young animals. The mechanisms underlying immunity to this parasite are not well understood (Schallig 2000).

This paper is translated from its Chinese version in *Scientia Agricultura Sinica*.
SUN Yan-ming, Ph D, Professor, Tel: +86-25-84395904, Fax: +86-25-84398669, E-mail: sym-2000@sohu.com; Correspondence LI Xiang-rui, Tel: +86-25-84395605, Fax: +86-25-84398669, E-mail: lixiangrui@njau.edu.cn

© 2007, CAAS. All rights reserved. Published by Elsevier Ltd.

Three novel tandem repeat-type galectin cDNAs from *H. contortus* have been documented (Greenhalgh *et al.* 2000). *Hco*-gal-1 contains an open reading frame (ORF) of 836 bp which encodes a predicted protein of 278 amino acids, while *Hco*-gal-3a, with an ORF of 831 bp, encodes a predicted protein of 277 amino acids. *Hco*-gal-3b has an ORF of 849 bp which encodes a protein of six more amino acids than *Hco*-gal-3a. *Hco*-gal-4 contains an ORF of 875 bp encoding a predicted protein of 291 amino acids. The levels of expression of these galectins differ in the life cycle of the parasites. *Hco*-gal-1 has been expressed at high levels in all parasitic stages, with a slightly higher expression in L_2 and unexsheathed L_3 (Greenhalgh *et al.* 2000). In contrast, *Hco*-gal-3a/b is maximally expressed in adult parasites, while *Hco*-gal-4 is upregulated in unexsheathed L_3 and its levels decline in adults. The function(s) of these galectins in parasitic nematodes remain unknown (Greenhalgh *et al.* 2000).

Although the majority of galectins act extracellularly, some intracellular functions have also been implied, including regulation of nuclear pre-mRNA splicing (Dagher *et al.* 1995), protection from apoptosis (Yang *et al.* 1996), and induction of apoptosis (Kuwabara *et al.* 2002). *Hco*-gal-m and *Hco*-gal-f galectins were cloned from male and female *H. contortus* previously (Li *et al.* 2005). However, the functions of them have not been established to date. According to Rabinovich *et al.* (1999), human recombinant galectin-1 was able to reduce the secretion of TNF-α and IFN-γ in a dose-dependent manner from which we made the hypothesis that recombinant *Hco*-gal-m/f would inhibit the cytokine mRNA transcription in goats. In this study, the effects of recombinant proteins of *Hco*-gal-m/f on the mRNA levels of IL-1β, IL-2, IL-4, IL-6, IFN-γ, and TNF-α of goat PBMCs *in vitro* were examined by the semi-quantitative reverse transcription RT-PCR technique.

MATERIALS AND METHODS

Materials

The reagents used in this study were obtained from various sources: concanavalin A (ConA) and lipopolysaccharides (LPS) (Sigma Chemical Co., USA); the avian myeloblastosis virus (AMV) reverse transcriptase, r*Taq* DNA polymerase, and related reagents (Takara Biotechnology Co., Ltd., Dalian, China); ficoll-hypaque, gradient centrifugation (Chuanye Co., Tianjin, China); heat-inactivated fetal calf serum (FCS, Sijiqing Biological Engineering Co., Ltd., Hangzhou, China); RPMI-1640 medium, Trizol, and DNase I (Invitrogen, USA). The recombinant *Hco*-gal-m/f proteins were prepared in the Laboratory of Veterinary Parasitology, Nanjing Agricultural University, China, as described by Li (2003). Its endotoxin amount was less than 0.5 EU mL^{-1} quantified by bacteria endotoxin test and stored at -20°C until use (Veterinary Pharmacopoeia Commission, People's Republic of China 2000).

Methods

Cell isolation and *in vitro* culture Heparinised blood samples were collected respectively by venipuncture of five healthy 2-year-old goats. The PBMCs were separated using standard ficoll-hypaque gradient centrifugation and washed twice in Ca^{2+}/Mg^{2+}-free phosphate-buffered saline (PBS, pH 7.4) and counted. Cell viability, as determined by trypan blue dye exclusion, was greater than 90% in all cases. PBMCs were diluted to 5×10^6 cells mL^{-1} in RPMI 1640 medium containing 10% heat-inactivated fetal calf serum, and supplemented with 100 U mL^{-1} penicillin, 100 mg mL^{-1} streptomycin, 2 mM-glutamine, and 5 mM 2-mercaptoethanol. Then 1 μg mL^{-1} LPS or 10 μg mL^{-1} ConA was added to the cell solution to induce IL-1β or the other cytokines. Cell suspensions were separated into five aliquots (2 mL/aliquot) in 24-well flat-bottomed culture plates (Costar, Cambridge, MA), and supplemented with recombinant *Hco*-gal-m or *Hco*-gal-f at concentrations of 0, 10, 20, 40, and 0 μg mL^{-1}, respectively. The first 0 μg mL^{-1} well served as control while the last 0 μg mL^{-1} well was used as 'no RT'-PCR control. Plates were incubated for different time ranges at 37°C in a humidified atmosphere containing 5% CO_2 until the cells were collected for RNA isolation.

RNA extraction After 4 h of culture incubation for IL-1β, 24 h for TNF-α, IL-2, IL-4, and IFN-γ, and 48 h for IL-6, cells were collected and washed, and the total cellular RNA were extracted from cells using TRIzol reagent. The RNA concentration was measured using

©2007, CAAS. All rights reserved. Published by Elsevier Ltd.

Download English Version:

https://daneshyari.com/en/article/4490748

Download Persian Version:

https://daneshyari.com/article/4490748

Daneshyari.com