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Mannose-binding lectin impairs *Leptospira* activity through the inhibitory effect on the motility of cell

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ABSTRACT

Mannose-binding lectin (MBL) plays key role in lectin pathway of innate immunity, and shows the ability of triggering opsonization intermediately. Substantial increase in the serum level of MBL has been confirmed during leptospirosis, which caused by a pathogenic spirochete, *Leptospira*. *Leptospira* has a fascinating locomotion pattern, which simultaneously gyrating and swimming forward, such motility enables that *Leptospira* is difficult to be captured by immune cells if without any assistance. In this study, the effect of mannose-binding lectin to *Leptospira* was quantitatively investigated by measuring some kinematic parameters, to discover the mechanism behind MBL-mediated immune responses during leptospiral infection. The results showed that mannose-binding lectin is capable of inhibiting the motility of *Leptospira* by transforming free swimming cells to tumbled rotating cells, resulted in the increase number of rotating cells. Otherwise, decrease in rotation rate of rotating cell has been observed. However, the swimming speed of swimming *Leptospira* cells showed no observable change under the effect of MBL. The inhibitory effect were only valid in a relatively short period, *Leptospira* cells regained their original motility after 2 h. This raises an interesting topic that *Leptospira* is somehow able to escape from the inhibitory effect of MBL by dragging such unfavorable molecules toward to the cell end and eventually throwing it out. The inhibitory effect of MBL on the motility of *Leptospira* is expected to provide a new insight into lectin pathway.

Introduction

Mannose-binding lectin (MBL) is a collagenous lectin found in the serum of mammals and avian (Turner 1996). It is believed to act an important role in innate immunity by binding to foreign microorganisms and then activating the complement system, via two MBL-associated serine proteases (MASP-1 and MASP-2) (Matsushita and Fujita 1992; Epstein et al. 1996; Thiel et al. 1997). Also, MBL is thought to directly interact with immune cells to promote the opsonization of bacteria in humans (Ezekowitz et al. 1989; Kuhlman et al. 1989).

MBL belongs to the protein family called collectins, which consists of collagenous domains and lectin domains (Epstein et al. 1996; Ip and Takahashi 2008). MBL is primarily synthesized in the liver, but can be detected at various sites. As other members of collectin family, MBL shows selective and calcium-dependent binding to terminal sugars D-mannose on the membrane of antigenic microbes (Drickamer 1992; Weis et al. 1992; Neth et al. 2000). Recent study reported the substantial increase of serum MBL level during the leptospirosis (Miranda et al. 2009).

Leptospirosis is a zoonotic disease caused by pathogenic strains of the spirochete *Leptospira* (Faine 1982). It has been reported worldwide, affecting accidental hosts, such as livestock, companion animals, and people, with mild to fatal symptoms (Bharti et al. 2003). The susceptible population includes citizens of impoverished areas and flood-borne regions of developing countries, farmers, sewer workers, and even tourists are susceptible population to this disease (McBride et al. 2005). The transmission of leptospirosis involves a *Leptospira* life cycle that has been adapted to include mammalian hosts, commonly, such as rat, in which the bacteria colonize the kidneys, shed in the urine. Susceptible hosts are infected via contact with contaminated water by

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dermal abrasions, mucous membranes, and conjunctivae (Sasaki et al. 1993; Haake et al. 2002).

Leptospira have periplasmic flagella beneath the outer cell membrane and show fascinating locomotion pattern. In externally flagellated bacteria such as *Escherichia coli* and *Salmonella* spp., their flagellar rotation generates thrust by interaction with fluids. In contrast, the role of *Leptospira* periplasmic flagella is not propelling the cell but transforming the cell body (Bromley and Charon 1979; Kan and Wolgemuth 2007; Nakamura et al. 2014). It has been known that the motile forms of *Leptospira* can be classified into two groups: swimming and rotating groups (Guo et al. 2012). *Leptospira* cells can smoothly swim when the anterior end is spiral shaped and the posterior end is hook shaped (Spiral–Hook). During swimming, the morphology of the cell body frequently changes. When both ends of the cell body are hook shaped (Hook–Hook) or spiral shaped (Spiral–Spiral), cells move neither forward nor backward and spin at one position (Goldstein and Charon 1988).

Bacterial motility is one of the essential pathogenic factor that enhances the invading ability and eventually leads bacteria to reach the target tissue. It has been known that applications of anti-flagella antibodies work on the inhibition of bacterial motility, thus sequentially attenuate corresponding diseases (Landsperger et al. 1994; Worley et al. 2006). Unfortunately, the utility of antisera targeting flagella has not been found in *Leptospira* because periplasmic flagella exist within the cell body. No effect of anti-flagella antibody on the motility of this spirochete has been also reported (Chang and Faine 1973). However, recent study showed antibody against outer membrane inhibits the motility of *Leptospira* (Guo et al. 2012), which provides a novel view and understanding of immune response to leptospiral infection. It is well known that *Leptospira* are readily opsonized and phagocytized by macrophages and neutrophils, while undergoing lysis once the complements are present (Adler and Faine 1978; Kuhlman et al. 1989; Masuzawa et al. 1996; Müller et al. 2010). However, there is no study on the inhibitory effect of MBL on the motility of *Leptospira*.

In this study, various kinematic parameters of the *Leptospira* motility, such as motile fraction, swimming speed, and rotation rate were measured under the affection of MBL, to identify the interrelation between MBL and motility. The results are expected to give a new insight into complement pathways in the response to leptospiral infection.

Materials and methods

Bacterial strain and cultural media

Leptospira biflexa serovar patoc strain Patoc I, and *Leptospira interrogans* serovar pomona strain Ponoma were used in the experiment. Mannose is known to be the sugar component of the lipopolysaccharide in Patoc I but not in Pomona (Matsuo et al. 2000). Cells were grown in Ellinghausen–McCullough–Johnson–Harris (EMJH) liquid medium (Johnson and Harris 1967) at 30 °C for 4 days (Patoc I) and 5 days (Pomona) respectively, to reach early logarithmic phase. Maintained in 10% normal rabbit serum (Gibco, Life Technologies, MD). The cells were then suspended in fresh EMJH medium, which was used as motility medium.

Measurement of motile fraction

The methods which are used to measure the motile fraction and swimming speed are described previously by Guo et al. (2012). Briefly, 4 days cultured *Leptospira* cells in the density of 1.0×10^8 cells/ml, were treated with human MBL (R&D Systems, Minneapolis, MN) in 2.5 μg/ml (Miranda et al. 2009) and otherwise the combination with 20 mM EGTA (Nacalai Tesque, Kyoto, Japan) buffer (Miller and Smith 1984), a commonly used chelator to bivalent calcium ions. Then observed under dark-field microscope (BH2; Olympus, Tokyo, Japan), the locomotion of bacteria was captured by a charge-coupled device camera (SSC-M350; Sony, Tokyo, Japan) at a frame rate of 30 fps and recorded by video recorder (WV-DR7; Sony, Tokyo, Japan). Appropriately captured DVD videos were converted to AVI movies, for further analysis.

Measurements of swimming speed and rotation rate

Swimming trajectory and speed of were analyzed by using software ImageJ (National Institutes of Health, Bethesda, MD) and Macros of Excel (Microsoft, Redmond, WA), which were developed from the previous study of (Nakamura et al. 2006).

To capture every single gyration of a rotating cell, High speed CCD camera (B0620; Imperx, FL, USA) was equipped to the dark-field microscope. The rotation in hook shaped end of rotating cell was intensively focused, appropriate parts of the video were captured on computer for further analysis.

At least four individual trials were carried out in each assay. Statistical analysis, such as significant test, Gaussian distribution was accomplished by using Microsoft Excel and Origin (OriginLab, Northampton, MA).

Results

Effect of MBL on Leptospira motility

We first observed that swimming *Leptospira* cells showed spiral shaped anterior ends and hook shaped posterior ends (Fig. 1a). When both ends of the cell body were spiral shaped or hook shaped, cells vigorously rotated, but they did not translate (Fig. 1b). These are consistent with previous reports (Charon et al. 1981; Goldstein and Charon 1988; Nakamura et al. 2014; Islam et al. 2014). Analysis of motile fractions in EMJH media showed that fractions of swimming and rotating cells were comparable, which were 45–50%, and some cells were non-motile (Fig. 1c).

To test the effect of MBL on the motility of *Leptospira*, we analyzed the time-dependent change in fractions of swimming and rotating cells in the presence or absence of MBL. No significant changes in swimming and rotating fractions were observed in the absence of MBL. When MBL was added to media, the fraction of swimming cells was found to decrease 10–30 min after starting assays (Fig. 2a). In contrast, the fraction of rotating cells was increased (Fig. 2b), suggesting that many, but not all, swimming cells were converted to rotating ones by addition of MBL. When MBL was treated with EGTA, the motility inhibition was not observed (Fig. 2a and b). This result supports our argument that the motility inhibition observed in this assay was caused by MBL, because the MBL binding to mannose is known to be dependent on bivalent calcium ion. We observed that the swimming fraction gradually recovered and eventually reached similar level to the control group after 2 h (Fig. 2a). The fraction of rotating cells showed an opposite tendency to that of swimming cells (Fig. 2b). These indicate the possibility that *Leptospira* can escape from MBL binding and regain their original motility.

Measurements of motile parameters

Swimming speeds were measured to test the affection of MBL on the swimming-mode cells more detail. The control groups that were analyzed in media without MBL showed the average speed was about 19.0 μm/s (Table 1). When cells were treated with MBL, their swimming speeds were slightly decreased (17.3 μm/s), but no significant differences were shown throughout the experiment