International Journal of Medical Microbiology 306 (2016) 212–221

Contents lists available at ScienceDirect

International Journal of Medical Microbiology

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

Mycobacterial antigen 85 complex (Ag85) as a target for ficolins and mannose-binding lectin

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ARTICLE INFO

Article history:
Received 13 November 2015
Received in revised form 7 March 2016
Accepted 25 April 2016

Keywords:
Mannose-binding lectin (MBL)
Ficolin
Lipoarabinomannan (LAM)
Antigen 85 (Ag85) complex
Mycobacterium

ABSTRACT

The pattern recognition molecules (PRMs) able to activate complement *via* the lectin pathway are suspected to be involved in the interaction between pathogenic *Mycobacteria* and the host immune response. Recently, we have found strong interactions between 25 and 35 kDa mycobacterial cell fractions and mannose-binding lectin (MBL) and ficolins. Here we demonstrate that two biologically important mycobacterial structures, mannosylated lipoarabinomannan (ManLAM) and the antigen 85 (Ag85) complex, induce activation of the lectin pathway of complement. The strong interaction of recombinant MBL with purified ManLAM was confirmed, but no binding of recombinant ficolins (ficolin-1, -2, -3) with this structure was observed. Interestingly, all PRMs tested reacted with the mycobacterial antigen 85 (Ag85) complex. Based on the use of specific inhibitors (mannan for MBL, acetylated bovine serum albumin for ficolin-1 and -2, *Hafnia alvei* PCM 1200 lipopolysaccharide for ficolin-3), we concluded that carbohydrate-recognition (MBL) and fibrinogen-like domains (ficolins) were involved in these interactions. Our results indicate that the mycobacterial antigen 85 complex is a target for ficolins and MBL. Furthermore, those PRMs also bound to fibronectin and therefore might influence the Ag85 complex-dependent interaction of *Mycobacterium* with the extracellular matrix.

1. Introduction

Mannose-binding lectin (MBL) and ficolins-1 (M-ficolin) -2 (L-ficolin) and -3 (H-ficolin) are pattern recognition molecules (PRMs) possessing the ability to activate complement *via* the lectin pathway (LP) and to contribute to the clearance of pathogens due to their opsonic properties. These proteins may also modulate the host immune response to microbial antigens (Wang et al., 2011; Bartlomiejczyk et al., 2014; Michalski et al., 2015; Bidula et al., 2015). Whereas MBL is a collectin, primarily recognizing sugar residues, the specificity of ficolins often involves acetyl groups of carbohydrate or non-carbohydrate compounds. Both collectins and ficolins possess a collagen-like triple helical region, responsible for complexing with mannose-binding lectin-associated serine proteases (MASPs), enzymes crucial for initiation of complement activation. However, collectins and ficolins differ by C-terminal domains, responsible for pattern recognition: collectins have a carbohydrate-recognition domain (CRD), but ficolins have a fibrinogen-like domain (FBG) (Thiel and Gadjeva, 2009).

The mycobacterial cell wall is known to be rich in glycoconjugates (polysaccharides, lipopolysaccharides, glycolipids and glycoproteins) that are potential targets for host complement activating collectins and ficolins. Recently, we have shown that MBL and ficolins agglutinate *Mycobacterium tuberculosis* (MTB) $H_{37}Rv$ cells and enhance their phagocytosis by macrophages. Interaction of those PRMs with lysates of mycobacterial cells led to complement activation (Bartlomiejczyk et al., 2014). The strongest reactivity was observed for 30–35 kDa fractions which may suggest the involvement of two cell wall components: lipoarabinomannan (LAM) and/or the antigen 85 (Ag85) complex. LAM is a

Abbreviations: ManLAM, mannosylated lipoarabinomannan; MBL, mannose-binding lectin (mannan-binding lectin); LAM, lipoarabinomannan; SPR, surface plasmon resonance; TRIFMA, time-resolved fluorimetric assay.

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http://dx.doi.org/10.1016/j.ijmm.2016.04.004
1438-4221/

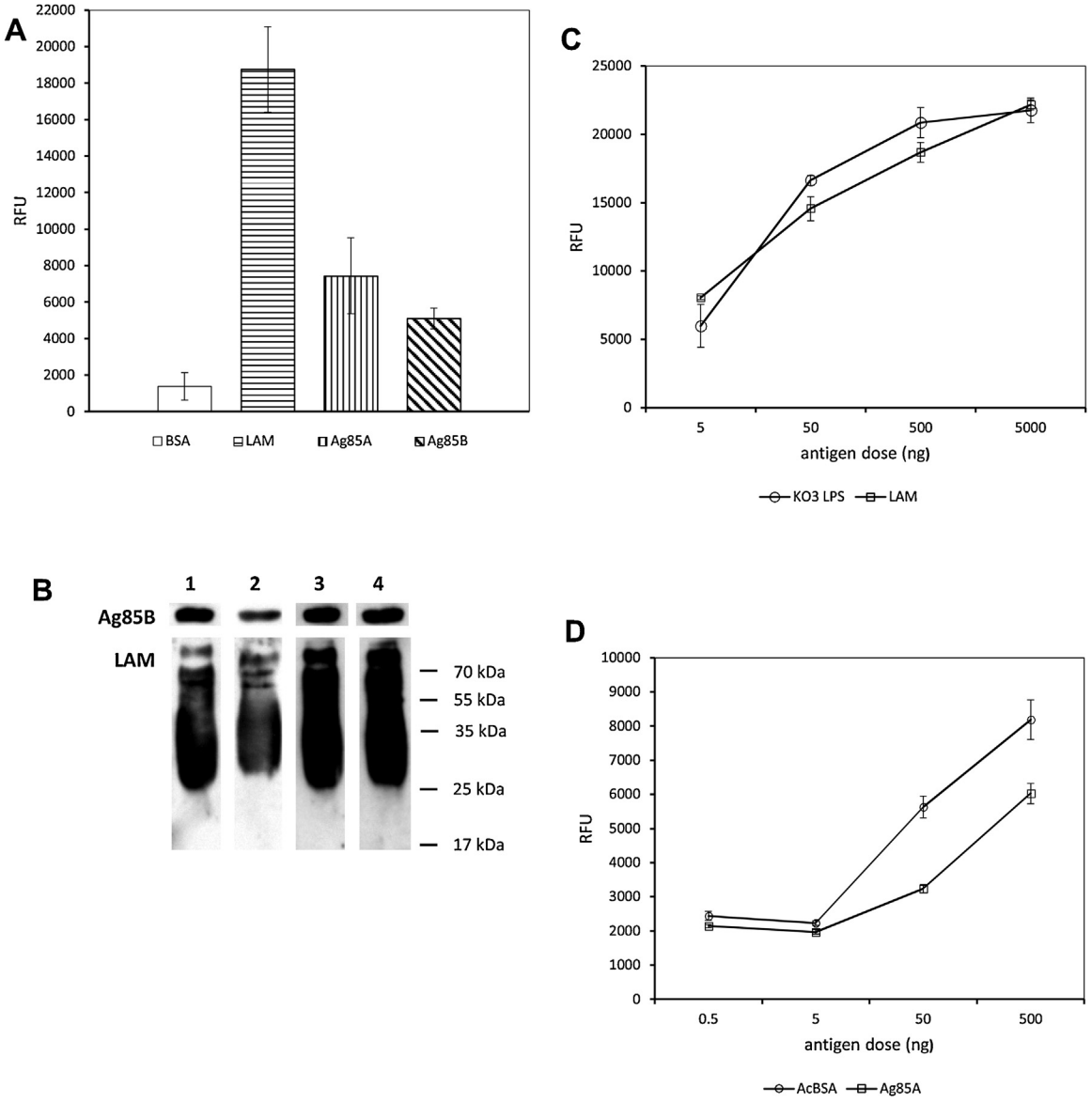

Fig. 1. Lectin pathway of complement-dependent C4 activation by ManLAM and Ag85 fractions. (A) data from TRIFMA (mean ± SD from 4 to 6 independent experiments). (B) Western blot (lane 1: membranes incubated with pooled NHS; lane 2: membranes incubated with pooled NHS pre-adsorbed with D-GlcNAc-agarose). Lanes 3 and 4 (corresponding to lanes 1 and 2, respectively) show the same membranes, after being stripped with 0.2 M glycine (pH 2.2) (to remove bound proteins/antibodies) and then developed again, with anti-Ag85B or anti-*M. tuberculosis* antibodies. That confirms that the amount of antigens used for demonstration of complement activation (lane 1) and its inhibition (lane 2) was the same; (C) the comparison of ManLAM- and *Klebsiella pneumoniae* O3 (KO3) LPS-dependent C4 activation (TRIFMA, data are shown as mean ± SD from at least 3 independent experiments); (D) The comparison of Ag85A- and AcBSA- (acetylated bovine serum albumin)-dependent C4 activation (TRIFMA, data are shown as mean ± SD from at least 3 independent experiments).

heterogeneous glycolipid, composed of a mannosyl-phosphatidyl-*myo*-inositol anchor, substituted with D-mannan and D-arabinan domains. In slow-growing, pathogenic mycobacteria (including MTB), the non-reducing terminal of D-arabinan is capped with D-mannose mono-, di- or trisaccharide thus forming mannosylated lipoarabinomannan (ManLAM). The latter is considered to be a mycobacterial virulence factor, facilitating the intracellular survival of bacteria by blocking maturation of phagosomes (Vergne et al., 2003; Kang et al., 2005; Torrelles et al., 2012). As a pathogen-associated molecular pattern molecule (PAMP), LAM was demonstrated to interact with some human pattern recognition molecules (PRMs), including those able to activate complement *via* the lectin pathway, like mannose-binding lectin, CL-LK (a complex of collectin-liver 1 with collectin kidney-1), or ficolin-2 (Polotsky et al., 1997; Luo et al., 2013; Bartlomiejczyk et al., 2014; Troegeler et al., 2015). Recently, Bahia El Idrissi et al. (2015) demonstrated involvement of LAM-dependent lectin pathway activation in peripheral nerve damage in leprosy (using a murine model). Lipoarabinomannan has been detected in serum, sputum and urine samples of tuberculosis (TB) patients (Sada et al., 1992; Pereira Arias-Bounda et al., 2000; Hamasur et al., 2001).

The mycobacterial 30–32 kDa antigen 85 complex comprises genetically distinct, but structurally/functionally related Ag85A, Ag85B and Ag85C proteins (Harth et al., 1996). They are either retained in the cell wall or secreted in a ratio of 2:3:1 (Bekmurzayeva et al., 2013). The members of Ag85 family possess activity of mycolyl transferases attaching mycolic acids to arabinogalactan or enabling generation of cord factor (TDM, trehalose dimycolate), important for the integrity of the mycobacterial cell wall and pathogenesis (Nguyen et al., 2005). Elamin et al. (2011) demonstrated also the activity of Ag85A as the diacylglycerol acyltransferase (DGAT) and suggested its participation in the formation