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Recombinant chimeric lectins consisting of mannose-binding lectin and L-ficolin are potent inhibitors of influenza A virus compared with mannose-binding lectin

Wei-Chuan Chang [a,1], Kevan L. Hartshorn [b,1], Mitchell R. White [b], Patience Moyo [a], Ian C. Michelow [a], Henry Koziel [c], Bernard T. Kinane [a], Emmett V. Schmidt [d], Teizo Fujita [e], Kazue Takahashi [a,*]

[a] Program of Developmental Immunology, Department of Pediatrics, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114, United States
[b] Department of Medicine, Boston University School of Medicine, Boston, MA 02118, United States
[c] Division of Pulmonary, Critical Care, and Sleep Medicine, Department of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA 02115, United States
[d] Cancer Center, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114, United States
[e] Department of Immunology, Fukushima Medical University, Fukushima 960-1295, Japan

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ABSTRACT

MBL structurally contains a type II-like collagenous domain and a carbohydrate recognition domain (CRD). We have recently generated three novel recombinant chimeric lectins (RCL), in which varying length of collagenous domain of mannose-binding lectin (MBL) is replaced with that of L-ficolin (L-FCN). CRD of MBL is used for target recognition because it has a broad spectrum in pathogen recognition compared with L-FCN. Results of our study demonstrate that these RCLs are potent inhibitors of influenza A virus (IAV). RCLs, against IAV, show dose-dependent activation of the lectin complement pathway, which is significantly higher than that of recombinant human MBL (rMBL). This activity is observed even without MBL-associated serine proteases (MASPs, provided by MBL deficient mouse sera), which have been thought to mediate complement activation. These observations suggest that RCLs are more efficient in associating with MASP-2, which predominantly mediates the activity. Yet, additional serum further increases the activity while RCL-mediated coagulation-like enzyme activities are diminished compared with rMBL, suggesting reduced association with MASP-1, which has been shown to mediate coagulation-like activity. These data suggest that RCLs may interfere less with host coagulation, which is advantageous to be a therapeutic drug. Importantly, these RCLs have surpassed rMBL for anti-viral activities, such as viral aggregation, reduction of viral hemagglutination (HA) and inhibition of virus-mediated HA and neuraminidase (NA) activities. These results are encouraging that novel RCLs could be used as anti-IAV agents with less side effect and that RCLs would be suitable candidates in developing a new anti-IAV therapy.

1. Introduction

IAV is an RNA virus whose surface is enveloped with glycoproteins containing neuraminidase (NA) and hemagglutinin (HA), which have glycosylation sites [1]. IAV infection is a common infection that could result in fatal complications, even in individuals who are appeared to be healthy [2,3]. Mortality and hospitalization are estimated to exceed annually 30,000 and 200,000, respectively in the United States alone [2]. Prevention is currently relied upon immunization, however vaccines are less effective in elderly and are not approved by the FDA for infants younger than 6 months of age [3,4]. Resistance to antiviral agents has developed in seasonal and pandemic IAV strains [2,5]. Thus, there is a need for new effective anti-IAV therapeutics.

The first line of host defense is the innate immunity, which recognizes pathogen-associated molecular patterns (PAMPs) through pattern recognition receptors and soluble molecules that include lectins [6]. One such lectin is MBL, which is primarily synthesized in the liver and circulates in the blood [7–9]. MBL belongs to the collectin family that is structurally characterized by consisting of a type II-like collagenous domain at N-terminus, followed by a neck region and a carbohydrate recognition domain (CRD) at C-terminus [10]. The collectin family includes lung surfactant protein (SP)-A and SP-D [11]. These surfactant proteins have anti-viral functions [12–17] and mice lacking SP-A or SP-D have increased susceptibility to AIV infection [14,18].

In early 1990, MBL was identified as a β-inhibitor, which had been discovered as an IAV inactivating serum factor in the 1940s

* Corresponding author at: 55 Fruit Street, GRJ1402, Boston, MA 02114, United States. Tel.: +1 617 726 1394; fax: +1 617 724 3248.
E-mail address: ktakahashi1@partners.org (K. Takahashi).
[1] Co-first authors.

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[19]. Since then, many studies have described MBL's anti-IAV functions, including inhibition of viral hemagglutination, inhibition of HA and NA and viral neutralization [20–23]. MBL activates complement via the lectin complement pathway, which is a key biologic function along with other complement pathways, such as the classical and the alternative complement pathway. The classical complement pathway is mediated by C1rs proteases, which are replaced by MASP-1, MASP-2 and/or MASP-3, in the lectin complement pathway [24]. Both pathways cleave C4 and C2 to generate the C3 convertase, C4bC2a [25,26].

MBL–MASP complex also initiates coagulation via thrombin-like activity [27,28]. Coagulation is a primitive yet effective host defense mechanism. For example, tachylectins in horseshoe crab hemolymph provide immune protection by clotting lipopolysaccharide and β-glucan, pattern recognition molecules of pathogens (PAMPs of Gram negative bacteria and fungus, respectively) [29].

The collectin family also includes L-FCN and H-FCN, which are also circulating serum pattern recognition molecules of innate immune system [30]. Like MBL, both FCNs contain the collagenous domain while CRD is replaced with fibrinogen-like domain, which preferentially recognizes acetylated molecules and sialic acid [31,32]. In contrast, MBL's target recognition is broad, including mannose, which is widely expressed on many pathogens [33]. It has been shown that other chimeric lectins consisting of MBL-CRD and the collagenous domain of SP-D gain anti-IAV activities, such as viral aggregation and inhibition of HA, NA and viral infectivity [22,34].

We have previously generated three RCLs consisting of L-FCN and MBL, in which various lengths of the collagenous domain were replaced with that of L-FCN [23]. Previous characterization study has demonstrated that these RCLs are either comparable to or surpassed rMBL for several biologic activities, including their binding to Nipah, Hendra and Ebola viruses [23]. Here, we further characterized biologic activities of these recombinant lectins against IAV using *in vitro* system and will discuss our findings.

2. Materials and methods

2.1. Recombinant chimeric lectins

Chimeric lectins were produced as previously described [23]. In this study, these lectins are named RCL1, RCL2 and RCL3, corresponding to L-FCN/MBL126, L-FCN/MBL76 and L-FCN/MBL64, respectively in the previous publication. All RCLs have MBL-CRD while MBL-collagenous domain was replaced with 126, 76 or 64 amino acids of L-FCN's collagenous domain, resulting in total amino acid length of 251, 255 or 254 for RCL1, RCL2 or RCL3, respectively. Thus, overall amino acid length is similar while RCL1 has the longest L-FCN collagenous domain followed by RCL2 and then RCL3. The junction of two proteins in RCL2 is located at the middle of a putative MASP-binding domain.

2.2. Virus preparations

IAV (A/Phillipines/82(H3N2)) was prepared as previously described [35]. Briefly, IAV was grown in the chorioallantoic fluid of chicken eggs and purified on a discontinuous sucrose gradient (Sigma–Aldrich, St. Louis, MO). Virus stocks were dialyzed against PBS (Sigma–Aldrich, St. Louis, MO) and aliquots were stored at −80 °C. HA titers were determined by titration with human type O, Rh^- red blood cells (RBCs) in PBS.

2.3. MBL binding assay

This assay was performed using previously described methods with a minor modification [36]. IAV concentration was arbitrary defined as 1000 U/ml, which was determined to be optimal for many *in vitro* studies based on dose response experiments. Briefly, 96 well plates were coated with mannan (Sigma–Aldrich, St. Louis, MO) or IAV and then blocked. Following wash, the wells were incubated with indicated concentrations of recombinant lectins. After wash, bound MBL was detected by mouse anti-hMBL monoclonal Ab (2A9, a gift from Dr. Gregory Stahl) [37], followed by alkaline-phosphatase conjugated anti-mouse Ab (Promega, Madison, WI) and pNTP substrate (Sigma–Aldrich, St. Louis, MO). Reaction was read at 415 nm using SpectraMax M5 (Molecular Devices, Sunnyvale, CA) and expressed as OD 415 nm reading. Assays were performed in triplicates and were repeated at least twice.

2.4. Mouse sera

MBL null mice were previously generated and fully backcrossed onto C57Black/6J [36,38]. Sera were collected and stored at −80 °C prior to the study. All animal experiments were performed under a protocol approved by the Subcommittee on Research Animal Care at Massachusetts General Hospital, Boston, MA.

2.5. Assays of the lectin complement activity

The lectin pathway assay was performed with a minor modification of previously described method [36]. Briefly, 96 well plates were coated with mannan or IAV as above. After wash and block, the wells were incubated with various concentrations of lectins with or without 1% MBL null sera (MASP source) diluted in a binding buffer, 10 mM Tris, pH 7.8, 10 mM $CaCl_2$, 1 M NaCl (all chemicals were purchased from Sigma–Aldrich, St. Louis, MO). After wash, the wells were incubated with human C4 and incubated at 37 °C. After wash, the wells were incubated with rabbit anti-hC4c Ab (Dako, Carpinteria, CA) followed by biotin-conjugated anti-rabbit Ab, alkaline phosphatase-conjugated biotin–avidin (ABC-AP system, Vector Labs, Burlingame, CA) and then with pNTP (Sigma–Aldrich, St. Louis, MO). The plates were read at 415 nm. Binding activity was expressed as OD 415 nm reading. Pooled human serum with known MBL concentration and C4 activity, which was arbitrarily defined as 1000 U/ml (State Serum Institute, Denmark), was used to generate a standard curve on mannan-coated wells. Assays were performed in triplicates and were repeated twice.

2.6. Assay of thrombin-like and factor Xa-like activities

These activities were assayed using previously described methods [39]. Briefly, 384 well plates were coated with mannan or IAV as above. After wash, the wells were incubated with various concentrations of lectins with or without 1% MBL null mouse serum or 1% MASP-1/3 null mouse serum (MASP source) [40] diluted in the binding buffer. After wash, wells were incubated with rhodamine 110-thrombin substrate (R22124, Invitrogen, Carlsbad, CA) or amino-4-methylcoumarin acetate (AMC)-factor Xa substrate (222F, American Diagnostica Inc., Stamford, CT) and read at 500 nm excitation/520 nm emission or 360 nm excitation/440 nm emission, respectively, using SpectraMax M5. The results were expressed as arbitrary units (AUs). Assays were performed in triplicates and were repeated twice.

2.7. Viral neutralizing assay

The assay was performed as previously described [41]. Briefly, viruses were pre-incubated with lectins and washed and then incubated with Madin-Darby Canine Kidney (MDCK) cells. Infection was assayed by FITC-conjugated anti-IAV antibody (Ab) (Millipore, Billerica, MA). Virus neutralizing activity as %inhibition