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Efficacy of recombinant chimeric lectins, consisting of mannose binding lectin and L-ficolin, against influenza A viral infection in mouse model study

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ABSTRACT

Influenza A virus infection could result in fatal complications. Although immunization is the most effective prevention it is not effective to pandemic infection and is less effective or not approved for certain age groups. Some influenza virus strains have developed resistance to antiviral agents. Thus, new therapeutic agents are urgently needed. We focused on innate immune molecules, including mannose-binding lectin (MBL). In order to optimize its antiviral activities, we have previously generated three recombinant chimeric lectins (RCL), by introducing portions of L-ficolin, another innate immune lectin. Our in vitro characterizations previously selected RCL2 and RCL3 for further investigations against viruses, including influenza viruses. Here, we examined efficacy of these lectins against infection with PR8 (H1N1) influenza A virus using mouse model studies and a human tracheal epithelial cell system. Our results provide in vivo evidence that RCL3 is effective agent against influenza virus infection. The therapeutic mechanisms are in part by providing host protective responses mediated by cytokines. We conclude that RCL3 is a potential new innate immune anti-influenza virus therapeutic agent.

1. Introduction

Infection with influenza virus, an RNA virus, is common and is normally self-resolving. However, influenza virus infection could result in fatal complications, even in individuals who are appeared to be healthy (Lynch and Walsh, 2007; Munoz, 2003). Mortality is estimated to exceed annually more than 30,000 in the United States alone (Lynch and Walsh, 2007). Prevention is currently relied upon immunization, however vaccines are less effective against pandemic infections. Immunization is also less effective in elderly and is not approved by the FDA for infants younger than 6 months old (Bouree, 2003; Munoz, 2003). Some seasonal and pandemic influenza viruses have already developed resistance to antiviral agents, like tamiflu (Lynch and Walsh, 2007; Saito et al., 2010). Thus, there is a need for new effective anti-influenza virus therapeutic and prophylactic agents.

The first line of host defense system is the innate immune mechanisms, including lectins, like MBL, which recognizes pathogens through carbohydrate recognition domain (CRD) (Ip et al., 2009). MBL, a serum protein, is present in lungs of healthy mice (Chang et al., 2010). Mice genetically lacking MBL are susceptible to infection with a common strain of Philippine 82 (H3N2), but are relatively resistant to a pandemic strain of H1N1 (pH1N1) influenza A virus (Chang et al., 2010; Ling et al., 2012). These results suggest that MBL is less effective against H1N1 influenza A virus infection and that optimization of MBL is required.

Therefore, we have previously generated three recombinant chimeric lectin (RCLs) by replacing various length of the collagenous domain of MBL with that of L-ficolin (Michelow et al., 2010). These RCLs are superior to MBL for several antiviral activities, including inhibition of hemagglutination and viral aggregation; and binding to other viruses, such as Nipah, Hendra and Ebola (Chang et al., 2010; Michelow et al., 2010). Importantly, all RCLs have reduced interference with the coagulation system. Such characteristic is a significant advantage as a therapeutic agent because infectious diseases can cause coagulation disorders (Nesheim, 2003).

Other important aspects of infectious disease outcome are host inflammatory responses, which are mediated by cytokines and are also modulated by lectins, including MBL (Chang et al., 2010; Moller-Kristensen et al., 2006). Uncontrolled inflammation due to infection cause tissue injury and obstruction while asymptomatic

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infection can be observed in commensalisms and symbiosis without illness (Casadevall and Pirofski, 2000).

Our previous studies selected RCL2 and RCL3 for further investigations (Chang et al., 2011). Here, we investigated efficacy of these recombinant lectins against PR8 (H1N1) influenza A virus infection using murine lung infection model studies and human tracheal epithelial cells, natural targets of influenza viruses in humans (van Riel et al., 2007).

2. Materials and methods

2.1. Recombinant chimeric lectins

Chimeric lectins were produced as previously described (Michelow et al., 2010). RCL2 and RCL3 corresponded to L-ficolin/MBL76 and L-ficolin/MBL64, respectively in our previous study (Chang et al., 2011; Michelow et al., 2010). In both RCLs, MBL-collagenous domain was replaced with 76 or 64 amino acids of L-ficolin's collagenous domain, resulting in total amino acid length of 255 or 254, respectively (Michelow et al., 2010). Recombinant human MBL was a gift from Enzon (Piscataway, NJ).

2.2. Virus preparations

Influenza A virus strain A/Puerto Rico/8/34 (PR8, H1N1) was prepared as previously described (Hartshorn et al., 2000). Briefly, PR8 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 −150 °C. Virus titers (fluorescent foci counts, ffc) were obtained by infection assay of Madin–Darby canine kidney (MDCK) cells (Hartshorn et al., 2000).

2.3. Mice

C57Black/6J mice were purchased from Jackson Laboratories (Bar Harbor, ME) and used at ages between 6 and 10 weeks old. All animal experiments were performed under a protocol approved by the Subcommittee on Research Animal Care at Massachusetts General Hospital, Boston, MA.

2.4. In vivo PR8 infection experiments

Infection experiments were performed as described previously (Chang et al., 2010). Briefly, mice were anesthetized with avertin (250 mg/kg, i.p.) and were then intranasally inoculated with 5×10^6 ffc of PR8 in 20 μl PBS. One hour after viral inoculation, mice were intraperitoneally injected with 75 μg of recombinant lectins or saline in 0.2 ml volume (Shi et al., 2004). The dose of 75 μg was chosen because it fully restored MBL functions in MBL knockout mice (Moller-Kristensen et al., 2006; Shi et al., 2004). Survival, clinical observations and body weight were recorded every 2–3 days till day 14. Survival curves were generated by the product-limit survival fit using JMP software (SAS Institute, Cary, NC).

Lung homogenates were prepared on day 3, 7 and 14 as previously performed (Chang et al., 2010). Briefly, lungs were harvested, weighed, and homogenized with 0.5 ml of PBS using a polytron tissue homogenizer. Homogenates were centrifuged and supernatants were stored in the −80 °C freezer.

2.5. In vitro human tracheal epithelial cell infection experiments

Human tracheal epithelial cells (ATCC, Manassas, VA) were plated at 4×10^4 cells/well in 100 μl of RPMI1640 media, supplemented with 10% fetal bovine serum in 48 well plates. After incubation for 1 h in a 5% CO_2 incubator the wells were washed with PBS. The wells were then incubated with PR8 (5×10^6 ffc/40 μl/well in RPMI1640), which were preincubated with recombinant lectins or PBS at 2 μg/ml in the CO_2 incubator for 1 h. 60 μl of culture media was added and incubated over night in the CO_2 incubator. Cell survival at 48 h was assayed using a WST8 cell counting kit (Dojindo Molecular Technologies, Inc., Bethesda, MD) by reading A_{450} with a Spectramax M5 plate reader. Cell survival (%) was calculated as media alone 100%. Supernatants and cell lysates were prepared at 24 and 48 h and stored in the −80 °C freezer.

2.6. Viral titrations by quantitative polymerase chain reaction (QPCR)

Total RNA from cell lysates, supernatants or lung homogenates were prepared using TRI agent (Sigma–Aldrich, St. Louis, MO), according to the manufacturer's instructions. For cell lysates and supernatants, 6 wells were pooled into duplicate (3 wells × 2 data points), in order to obtain sufficient materials. cDNA was prepared using iScript cDNA synthesis kit (Bio-Rad, Hercules, CA). The RT-PCR primers and the probe were designed specific to H1N1 and were: sense, 5′-CCAGGAAATGCTGAGATCGAAGAT-3′; antisense, 5′-GGCAAGACTTGTGAGCAACTGA-3′; the probe, 5′-CACGGTCTGCACTCAT-3′, the latter was labeled with a reporter dye 6-carboxyfluorescein. PCR was performed in the Realplex^2 thermal cycler (Eppendorf North America, Hauppauge, NY). PCR program was one cycle of 95 °C for 10 min followed by 40 cycles of 95 °C for 15 s then 60 °C for 1 min. Standard curves were generated from serially diluted virus cDNA with known viral titers in each experiment. PR8 particles were normalized to the total RNA (μg).

2.7. Cytokine assays

Three supernatants of human tracheal cell culture were pooled in each test group to have sufficient sample volumes. Similarly, supernatants of lung homogenates from three mice were pooled. These samples were assayed using membrane array kits (human cytokine array C-series 4000 for 274 cytokines and mouse cytokine array C-series 2000 for 144 cytokines) (Raybiotech Inc., Norcross, GA). Developed membranes were scanned using a Chemi-Doc scanner (Bio-Rad, Horcuris, CA). Relative chemiluminescence intensity of each spot, corresponding to each molecule, was recorded using the Quantity One software provided by the scanner. Arbitrary units for each molecule were derived by normalizing the relative intensity against an average of the positive controls in each membrane (supplemental data). Using these arbitrary units, fold changes were calculated against those from control lungs (no viral infection). Positive molecules were defined as those with more than 3-fold increase at any time point.

2.8. Statistical analysis

All data were analyzed for statistical significance using JMP software. *P* values less than 0.05 were considered to be significant and no values were provided where there was no statistical significance. Statistical methods applied were indicated in each figure legend.

3. Results

3.1. RCL3 improves PR8 infection

Recombinant lectins were examined for therapeutic efficacy against PR8 infection using a murine lung infection. Without infection, all mice survived following administrations of recombinant lectins or saline. In contrast, upon viral infection, mean survival time was 8.7, 9.4, 11.1, and 11.9 days for saline control, MBL, RCL2,