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Adjuvant effects of mannose-binding lectin ligands on the immune response to infectious bronchitis vaccine in chickens with high or low serum mannose-binding lectin concentrations

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ABSTRACT

Mannose-binding lectin (MBL) plays a major role in the immune response as a soluble pattern-recognition receptor. MBL deficiency and susceptibility to different types of infections have been subject to extensive studies over the last decades. In humans and chickens, several studies have shown that MBL participates in the protection of hosts against virus infections. Infectious bronchitis (IB) is a highly contagious disease of economic importance in the poultry industry caused by the coronavirus infectious bronchitis virus (IBV). MBL has earlier been described to play a potential role in the pathogenesis of IBV infection and the production of IBV-specific antibodies, which may be exploited in optimising IBV vaccine strategies. The present study shows that MBL has the capability to bind to IBV *in vitro*. Chickens from two inbred lines (L10H and L10L) selected for high or low MBL serum concentrations, respectively, were vaccinated against IBV with or without the addition of the MBL ligands mannan, chitosan and fructooligosaccharide (FOS). The addition of MBL ligands to the IBV vaccine, especially FOS, enhanced the production of IBV-specific IgG antibody production in L10H chickens, but not L10L chickens after the second vaccination. The addition of FOS to the vaccine also increased the number of circulating CD4+ cells in L10H chickens compared to L10L chickens. The L10H chickens as well as the L10L chickens also showed an increased number of CD4−CD8α−γδ T-cells when an MBL ligand was added to the vaccine, most pronouncedly after the first vaccination. As MBL ligands co-administered with IBV vaccine induced differences between the two chicken lines, these results indirectly suggest that MBL is involved in the immune response to IBV vaccination. Furthermore, the higher antibody response in L10H chickens receiving vaccine and FOS makes FOS a potential adjuvant candidate in an IBV vaccine.

Introduction

The first line of host defence against pathogens involves the innate immune system. Pathogens have specific microorganism-associated molecular patterns (MAMPs) that are recognised by pattern-recognition receptors (PRRs). Two kinds of PRRs exist: surface PRRs and soluble PRRs. PRRs trigger intracellular signalling cascades upon MAMP recognition culminating in activation of antigen presenting cells and production of co-stimulatory molecules as well as pro-inflammatory cytokines. The production of co-stimulatory molecules and pro-inflammatory cytokines initiates the early host response to infection and also partakes in the activation and shaping of the adaptive immune response (Crozat et al. 2009; de Visser and Coussens 2005; Hoffmann et al. 1999). Several soluble PRRs have been described, and an example of such is mannose-binding lectin (MBL). MBL has various functions in, for example, complement activation, promotion of complement-independent opsonophagocytosis, modulation of inflammation, recognition of altered self-structures and apoptotic cell clearance (Dommett et al. 2006). MBL is a collectin consisting of multiple identical polypeptide chains oligomerised into different sizes. The chains are made up of four distinct domains. These are a cysteine-rich N-terminal domain, a collagenous domain, a neck domain, and a calcium-dependent carbohydrate-recognition domain (CRD) at the C-terminal (Takahashi 2011). It is the CRD that permits MBL

Abbreviations: CRD, carbohydrate-recognition domain; CTL, cytotoxic T-lymphocyte; FOS, fructooligosaccharide; IB, infectious bronchitis; IBV, infectious bronchitis virus; MBL, mannose-binding lectin; OPA, oropharyngeal airway; MAMP, microorganism-associated molecular patterns; PBS+, Gibco® DPBS with 0.1% BSA; PRR, pattern-recognition receptors; PV, post vaccination; qRT-PCR, real-time quantitative reverse transcription PCR; SARS, severe acute respiratory syndrome; SAv-HRP, Streptavidin Horseradish Peroxidase; TMC, N,N,N-trimethylated chitosan.

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to bind in a Ca^{2+}-dependent manner to MAMPs such as polysaccharides. In fact, terminal sugars, such as D-mannose, L-fucose and N-acetyl-D-glucosamine, found on the surface of many microorganisms, contain equatorial 3- and 4-hydroxyl groups to which MBL binds. MBL does not bind to D-galactose and sialic acid found on the surface of many animal cells. Beside sugars, MBL has also been found to bind to phospholipids, nucleic acids, and non-glycosylated proteins (Ip et al. 2009). The MBL genes in human (Madsen et al. 1994, 1995; Steffensen et al. 2000; Sumiya et al. 1991), porcine (Juul-Madsen et al. 2011a; Lillie et al. 2007), bovine (Liu et al. 2011) and chickens (Kjaerup et al. 2013) have been found to have polymorphisms resulting in wide variations in MBL serum levels in the organisms.

Over the last decades MBL deficiency and the influence on the susceptibility to different types of infections have been subject to extensive studies as reviewed (Heitzeneder et al. 2012; Mayilyan 2012; Takahashi 2011). Some results indicate that MBL deficiency may actually be beneficial with regard to disease, for example visceral leishmaniasis (Santos et al. 2001). However, most results suggest that MBL deficiency leads to a weaker immune response. In humans, several studies have shown that MBL participates in the protection of hosts against virus infections, such as infections with influenza A virus (Chang et al. 2010), Hepatitis C virus (Brown et al. 2010), Ebola virus (Michelow et al. 2011), and severe acute respiratory syndrome (SARS) coronavirus (Ip et al. 2005; Zhou et al. 2010). Thus, MBL in chickens may also play a role in the pathogenesis of chicken virus infections and the production of antibodies as suggested by Juul-Madsen et al. (2007).

Selective breeding of chickens for low or high serum MBL concentrations has been performed for several generations at our department as published by Juul-Madsen et al. (2007). This has resulted in two distinct chicken lines designated high (L10H) or low (L10L) with mean serum MBL concentrations of 33.4 μg/mL serum (L10H) and 7.6 μg/mL serum (L10L) (F14 generation, unpublished). Studies using these chicken sublines as well as outbred chickens have shown an inverse relationship between the MBL concentrations and the pathogen-specific antibody response (Juul-Madsen et al. 2007; Schou et al. 2008). Studies in mice have shown that MBL deficiency may result in a higher IgG antibody response after infections (Carter et al. 2007) and vaccinations (Guttormsen et al. 2009). From these results it can be hypothesised that basal MBL plasma levels may influence specific humoral immune responses. This explanation for this may be that either: (1) MBL pushes the immune response into a more cellular response (Th1 vs. Th2); (2) MBL efficiently neutralises the pathogen via the complement membrane-attack complex and no adaptive immune response is needed; or (3) MBL influences the pro-inflammatory cytokine production via interaction with surface receptors, such as toll-like receptors (Ip et al. 2008).

Infectious bronchitis (IB) is a highly contagious disease of economic importance in the poultry industry with symptoms such as sneezing, tracheal rales, and coughing. Furthermore, IB may cause a decline in egg quality and production in layers (Raj and Jones 1997). IB is caused by the coronavirus infectious bronchitis virus (IBV) which is highly able to genetically mutate and recombine. As a result, there is a continuous development of new strains throughout the world. Different strains can co-circulate within a region, and the severity of the disease varies from strain to strain and from flock to flock (Capua et al. 1999; Cavanagh 2007; Cook et al. 2012). Consequently, applied vaccines sometimes provide insufficient protection, as vaccination with one strain of IBV may not be protective against other strains.

Vaccine efficacy may be improved by the use of adjuvants. Good candidates for vaccine adjuvants are carbohydrates since they are mostly of low toxicity and high biocompatibility and furthermore play major roles within the immune system (Petrovsky and Cooper 2011). Carbohydrates such as mannan (Liu et al. 2012), chitosan (Rauw et al. 2010), and fructooligosaccharide (FOS) (Benyacoub et al. 2008) have previously been used in vaccines or diets as modulators of the immune response. These three carbohydrates are potential MBL ligands owing to their content of sugar units. The hypothesis of this study was that immunity after IBV vaccination may be improved after temporarily inhibition of the MBL function. This was achieved by adding an MBL ligand (mannan, chitosan, or FOS) to the vaccine given to chickens and thereby creating an artificial MBL deficiency during vaccination. Innate as well as adaptive immunological parameters were measured throughout the experimental period.

Materials and methods

Chemicals and reagents

All chemicals were obtained from Sigma–Aldrich, Ballerup, Denmark, except when noted. DreamTaq™ Master Mix was obtained from Qiagen, and oligonucleotide primers and probes were obtained from Eurofins MWG Operon, Ebersberg, Germany.

Purified MBL

Purified chicken MBL was bought from the Department of Cancer and Inflammation Research, University of Southern Denmark. It was purified from chicken serum as previously described (Laursen et al. 1998a).

MBL-IBV binding assay

The binding capacity of MBL to IBV was measured using ELISA. Dilutions of purified MBL and serum samples from L10L or L10H chickens were made with or without addition of saccharides. BioWhittaker® Veronal buffer (Lonza, Walkersille, MD, USA; cat. no. 12-624E) adjusted to a final concentration of 5 mM $MgCl_2$ and 10 mM $CaCl_2$ was used for diluting the samples. For the titration of MBL binding, concentrations of 0, 1, 2, 4, 8, 16, 24 and 32 μg/mL purified MBL were used, and 5 μL serum samples were diluted 1:50. The concentrations of the saccharides added were as follows: mannan 100 mg/mL; FOS 100 mg/mL; chitosan 10 mg/mL; galactose 9 mg/mL; and EDTA 20 mM. One-hundred microlitres of the dilutions and saccharides were mixed in a Nunc 96-well polypropylen MicroWell plate (Thermo Fisher Scientific, Slangerup, Denmark; cat. no. 442587) and incubated for 5 min before the samples were transferred to a 96-well microtitre plate coated with IBV antigen (from The ProFLOK® IBV Antibody Test Kit from Synbiotics Corporation, San Diego, CA, USA; cat. no. 96-6506). Wells receiving only buffer were used as negative controls. All dilutions were added in triplicates. The plate was then incubated at room temperature for 1 h. After a washing step with Gibco® DPBS supplemented with calcium and magnesium (Life Technologies Europe BV, Nærum, Denmark; cat. no. 14080-048) and pH adjusted to 7.4 followed by supplementation with 0.1% BSA (hereafter called PBS+), the wells were incubated for 45 min at room temperature with 1 μg/mL of biotinylated monoclonal mouse anti-cMBL (BioPorto Diagnostics A/S, Gentofte, Denmark; cat. no. HYB 182-01) in PBS+. After another washing step Streptavidin Horseradish Peroxidase (SAv-HRP) (BD Biosciense, Albertslund, Denmark; cat. no. 554066) diluted 20,000-fold in PBS+ was added. After 30 min of incubation and washing with PBS+, the presence of SAv-HRP was detected by adding 100 mL of substrate solution (<0.05%, wt/wt; 3,3′,5,5″ tetramethylbenzidin). Colour development was stopped with a 1 M H_2SO_4 solution. The colour development was determined by