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Glycans from avian influenza virus are recognized by chicken dendritic cells and are targets for the humoral immune response in chicken

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ABSTRACT

To increase our understanding of the interaction between avian influenza virus and its chicken host, we identified receptors for putative avian influenza virus (AIV) glycan determinants on chicken dendritic cells. Chicken dendritic cells (DCs) were found to recognize glycan determinants containing terminal αGalNAc, Galα1-3Gal, GlcNAcβ1-4GlcNAcβ1-4GlcNAcβ (chitotriose) and Galα1-2Gal. Infection of chicken dendritic cells with either low pathogenic (LP) or highly pathogenic (HP) AIV results in elevated mRNA expression of homologs of the mouse C-type lectins DEC205 and macrophage mannose receptor (MMR), whereas expression levels of the human dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) homolog remained unchanged.

Following uptake and subsequent presentation of avian influenza virus by DCs, adaptive immunity, including humoral immune responses are induced. We have investigated the antibody responses against virus glycan epitopes after avian influenza virus infection. Using glycan micro-array analysis we showed that chicken contained antibodies that predominantly recognize terminal Galα1-3Gal-R, chitotriose and Fucα1-2Galβ1-4GlcNAc-R (H-type 2). After influenza-infection, glycan array analysis showed that both levels and repertoire of glycan-recognizing antibodies decreased. However, analysis of the sera by ELISA indicated that the levels of different isotypes of anti-glycan Abs against specific glycan antigens was increased after influenza-infection, suggesting that the presentation of the glycan antigens and iso-type of the Abs are critical parameters to take into account when measuring anti-glycan Abs. This novel approach in avian influenza research may contribute to the development of a broad spectrum vaccine and improves our mechanistic understanding of innate and adaptive responses to glycans.

1. Introduction

The molecular events leading to glycan-induced innate and adaptive host immune responses are far from understood, but involve the innate immune system that recognizes the pathogen via pathogen recognition receptors on antigen-presenting cells (Van Kooyk and Rabinovich, 2008). Glycans present on the surface of pathogens can be recognized by lectins, carbohydrate-binding proteins that are either soluble or membrane-bound. Heavily glycosylated influenza viruses are therefore susceptible to capture by the host innate immune system (Vigerust and Shepherd, 2007; Vigerust et al., 2007). Various classes of lectins exist, including the galectins, the Ca^{2+}-dependent C-type lectin receptors (CLRs) and siglecs (reviewed by Van Kooyk and Rabinovich, 2008). In the chicken several types of soluble lectins are described, e.g. collectins including mannan-binding lectin, and chicken lung lectin (Hogenkamp et al., 2008; Laursen et al., 1998; Reemers et al.,

Abbreviations: Ab, antibody; AIV, avian influenza virus; (BM)-DC, (bone marrow-derived) dendritic cell; BPL, beta-propiolactone; CLR, C-type lectin; DC-SIGN, dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin; HA, haemagglutinin; HP, highly pathogenic; HRP, horseradish peroxidase; Ig, immunoglobulin; LP, low pathogenic; MGL-1, macrophage galactose-type lectin 1; MMR, macrophage mannose receptor; NA, neuraminidase; NGS, N-glycosylation sites; PAA, polyacrylamide; SA, sialic acid.

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2010), but limited information is available on membrane-bound lectins. DEC205, an endocytic receptor belonging to the CLR family is expressed by chicken bone marrow-derived dendritic cells (BM-DCs; Wu et al., 2010) and a dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN)/CD209 homolog was identified in the chicken based on sequence homology (Lin et al., 2009). In mice it was shown that the macrophage mannose receptor (MMR) and also the macrophage galactose-type lectin 1 (MGL-1) bind to haemagglutinin (HA) or neuraminidase (NA) on influenza A virus to facilitate uptake and destruction of the virus by macrophages (Reading et al., 2000; Upham et al., 2010). In addition, CHO cells deficient in sialic acid (SA) and transfected with DC-SIGN or L-SIGN can mediate attachment and entry of influenza virus. Treatment of these transfected cells with mannan blocked infection, a finding consistent with SA-independent infection via CLR (Londrigan et al., 2011).

Most viruses use the host cell glycosylation machinery for glycosylation of their glycoproteins (Olofsson and Hansen, 1998). Although the glycan structure is specified by the host cell, a general increase in the level of glycosylation of viral proteins is often observed and glycan profiles of virus proteins can therefore be distinguished from self glycosylated proteins and lipids (Vigerust and Shepherd, 2007). In addition, certain virus glycans, such as terminal oligomannosidic structures are not generally found on host glycoproteins (Vigerust and Shepherd, 2007). Furthermore, NA can remove sialic acids from glycans expressed on HA (Basak et al., 1985). There is extensive variation in the number and also location of potential glycosylation sites on the globular head of HA between different subtypes (Inkster et al., 1993) and different strains (Abe et al., 2004; Ward and Dopheide, 1981) of influenza A viruses. The importance of glycans on the head and stem region of HA and on NA for viral infectivity has been described (Chen et al., 2008; Deshpande et al., 1987; Gunther et al., 1993; Li et al., 1993) and these glycans also play a role in induction of anti-viral immunity via their interaction with several types of lectins, glycosylation may either shield conserved epitopes on HA (Das et al., 2010), or act as a target for carbohydrate-binding agents such as collectins (reviewed by Ng et al., 2012), galectins (Chernyy et al., 2011) and CLR (Londrigan et al., 2011; Reading et al., 2000; Upham et al., 2010). In the chicken, chicken lung lectin inhibited the hemagglutination activity of the human IAV virus isolates Phil/82 (H3N2; highly glycosylated HA) and PR8 (H1N1; poorly glycosylated HA; Hogenkamp et al., 2008).

It is well established that the humoral immune response plays an important role in controlling influenza virus infections (Duncker, 1972, 1974; Suarez and Schultz-Cherry, 2000; Toth and Siegel, 1993) and the induction of neutralizing antibodies (Ab) is a read out for vaccine efficacy (Sarawar et al., 1994). Ab are mainly directed against the highly variable surface proteins HA and NA and part of these Ab are directed against glycan structures on HA (Legastelois et al., 2011). Although the surface proteins are highly variable, the essential glycans present on the stalk of HA might be attractive targets for the generation of broad spectrum and heterosubtypic immunity (Wang et al., 2009).

The induction of protective Ab responses can be affected by the glycosylation status of the surface glycoproteins of viruses as shown for influenza A virus (Skehel et al., 1984; Wang et al., 2009; Wanzeck et al., 2011), human immuno deficiency virus (Quinones-Kochs et al., 2002), hepatitis C virus (Helle et al., 2010) and Newcastle disease virus (Panda et al., 2004), with poorer anti-protein Ab responses against more heavily glycosylated viruses. However, humoral responses against glycans have also been described against a range of pathogens, from viruses to nematodes (Pejchal et al., 2011; Van Stijn et al., 2010; Vervelde et al., 2003) and may contribute to protective immunity.

Knowledge about immune responses against glycan epitopes can assist the development of new vaccines. To increase our understanding of the mechanisms by which the immune responses against AIV in chickens are shaped and how we can induce broad spectrum immunity against different viral subtypes, we studied glycosylation patterns of different AIV strains since glycans that are shared by different HA subtypes could be of use in the development of a broadly cross-protective vaccine. Studying these glycans may also help to unravel immune evasion by AIV.

We studied the binding of chicken DCs to putative AIV glycans. Changes in CLR expression were studied after infection of chicken DCs with related low or highly pathogenic AIV strains and after culture with the same inactivated strains. Finally, we have investigated the Ab responses against glycan epitopes in serum after infection.

2. Materials and methods

2.1. Chickens

Lohmann Brown chickens were purchased from Verbeeks Broederij BV (Renswoude; the Netherlands). Animals were housed in groups and received food and water ad libitum. In compliance with Dutch law, all experiments were approved by the Animal Experimental Committee of the Faculty of Veterinary Medicine of Utrecht University, the Netherlands, in accordance with the Dutch regulation on experimental animals.

2.2. Influenza viruses

Chickens were infected with low pathogenic (LP) H9N2 A/Chicken/Saudi Arabia/SP02525/3AAV/2000 (H9N2), purchased from Animal Health Service, Deventer, the Netherlands. DCs were infected with the LP AI virus isolate H7N1 A/Chicken/Italy/1067/99 (LP H7N1) or with the highly pathogenic (HP) AI virus isolate H7N1 A/turkey/Italy/4580/99 (HP H7N1), both kindly provided by Drs. I. Capua and W. Dundon. All viruses were propagated in SPF embryonated chicken eggs. Work with HP H7N1 was performed in a biological safety level 3 (BSL3) facility. Experiments with LPAI viruses were performed in a BSL2 facility. For use in lectin spotblot and for DCs culture, viruses were inactivated using beta-propiolactone (BPL; Acros organics) and concentrated by ultracentrifugation as previously described (De Geus et al., 2011, 2012). Protein content was determined using a BCA protein assay (Pierce). The number and position of potential N-linked glycosylation sites (NGS) on HA and NA of the different AIV strains was determined by enumerating the tripeptide sequences N-X-S or N-X-T, where X can be any amino acid except Pro, using the N-GlycoSite tool (Zhang et al., 2004). H9N2 HA was sequenced by GD Animal Health Service, NA was sequenced by BaseClear B.V. Sequences of HA (GenBank: ACZ48601.1) and NA (GenBank: CAC95055.1) of LP H7N1 were found at the NCBI Influenza Virus Database.

2.3. Avian influenza virus infection of chickens

Eight-wk-old chickens were inoculated with 0.1 ml solution intranasally and 0.1 ml solution intratracheally containing 1×10^6 EID50/ml H9N2. Serum samples were collected before and 14 days post infection (p.i.). Before use in ELISA, samples were heat-inactivated for 30 min at 56 °C.

2.4. Lectin spotblot

To determine glycosylation patterns of AIV, BPL-inactivated and concentrated virus was spotted on nitrocellulose sheets. After drying, sheets were blocked with PBS/2.5% Protifar for 2 h at room