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THE ROLE OF NEURAMINIDASE IN THE PATHOGENICITY OF NEWCASTLE DISEASE: A REVIEW

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SUMMARY

The enzyme neuraminidase (mucopolysaccharide N-acetylneuraminyl hydrolase EC 3.2.1.18) is part of haemagglutininneuraminidase protein present in Newcastle disease virus (NDV) and all members of paramyxovirus genus. Neuraminidases are known to play an important role in the pathogenicity of many diseases by enzymatic removal of sialic acids from carbohydratecontaining molecules, such as erythrocytes of chickens and other animal species. It is also believed that neuraminidases could facilitate the production of infectious particles *in vitro* by removing sialic acid residues, and exposing appropriate cleavage site in cell culture. This special feature will enable neuraminidases to fulfill important pathological role during infection or disease. Because of the presumed role of neuraminidases in pathogenicity of diseases, it is important to critically examine the neuraminidase of NDV in relation to its intimate connection with the structure and function of the host cells, and the often serious consequences that result during and after NDV infection in poultry.

KEY WORDS: Neuraminidase, Newcastle disease, Pathogenicity, Sialic acids, Review

INTRODUCTION

Newcastle disease virus (NDV) is the causative agent of a major poultry disease in the world. The virus has a wide range of susceptible avian hosts. About eight thousand species from twenty-seven of the fifty orders of birds are apparently susceptible to NDV (Kaleta and Baldauf, 1988). The susceptibility of different avian hosts to NDV has been demonstrated in both naturally occurring and experimentally induced infections (Roy *et al.*, 2000; Wehmann *et al.*, 2003).

Newcastle disease (ND) is still dreaded and causes serious economic losses in poultry industry in many parts of the world, owing to its high mortality and morbidity rates (Oladele *et al.*, 2003; Saidu *et al.*, 2006). For example, infection of susceptible birds with virulent strains of NDV could result into morbidity and mortality of about 50% and 100%, respectively. Egg production may be reduced drastically or complete loss of egg production may be experienced in infected flock following infection with virulent strains of the virus (Alexander, 1997; Aldous *et al.*, 2003; Al-Garib *et al.*, 2003). Reports from many parts of Nigerian rate ND as one of the greatest constraints to the development of rural poultry production (Adene, 1990) Also among the

diseases of poultry, ND constitutes the most important epizootic disease in most developing countries, causing serious economic threat to poultry (Shamaki *et al.*, 1989; Oladele *et al.*, 2003).

DISTRIBUTION OF NEWCASTLE DISEASE VIRUS HAEMAGGLUTININ-NEURAMINIDASE (HN) PROTEIN

Newcastle disease virus contains six proteins (Samson, 1988; Gould *et al.*, 2003). One of the most important of these proteins is the haemagglutinin-neuraminidase (HN) protein, which is responsible for haemagglutinin and neuraminidase activities of the virus (McGinnes and Morrison, 1986; Lin *et al.*, 2003). It is known that both the haemagglutinin and neuraminidase activities are found on larger NDV glycoprotein in contrast to the distribution of these activities on two separate glycoproteins in orthomyxoviruses (Gould *et al.*, 2003; Kommers *et al.*, 2003). By analogy with orthomyxoviruses, the haemagglutination activity is a consequence of the adsorption of virus to cell via the virus glycoprotein and cell surface receptors (Lipkind and Shimanter, 1986). These receptors contain sialic (neuraminic) acid. One of the presumed roles of the neuraminidase activity is to aid elution of budding virons from the host cell by destroying local receptors. Sialic acid residues are not found on glycoproteins from virons which contain neuraminidase and this is thought to be significant in preventing dissemination (Alexander *et al.*, 1999).

In some avirulent NDV strains, such as Queensland V4 and Ulster 2C, the HN protein is synthesized as an inactive precursor HN_0 (Gould *et al.*, 2003). The NDV HN gene sequence studies have revealed that there is a single open reading frame coding for 577 amino acids for both NDV Beaudette C and Hitchner B1 strains, with predicted unglycosylated molecular weight of 63,149 and 63,250 daltons, respectively. Both strains contain a highly hydrophobic sequence close to the N terminus, which is highly conserved between the two strains (Russell *et al.*, 1990; Gould *et al.*, 2003).

Neuraminidases cleave the O-glycosidic linkages between the terminal sialic acids and the sub-terminal sugars of the free and glycoconjugates-bound oligosaccharides as one of the first steps in sialoglycoconjugate degradation. Neuraminidases are also present in metazoan animals and in diverse microorganisms, such as viruses, fungi, bacteria and protozoan parasites (Guzman *et al.*, 1990; Engstler *et al.*, 1993). In view of their ability to cleave O-glycosidic linkages, it is believed that these enzymes play a major role in spreading infection or acting as virulence factor in invasive infections (Godoy *et al.*, 1993; Kommers *et al.*, 2003).

THE DISTRIBUTION OF SIALIC ACIDS RECEPTORS IN NEWCASTLE DISEASE VIRUS AND OTHER ORGANISMS

In general, sialic acids have great chemical and biological diversity. They are ubiquitous, relatively large, hydrophilic and acidic molecules that exert physicochemical effects on glycoconjugates to which they are bound, and on the environmental molecules *in situ*; for example, in cell membrane (Schauer *et al.*, 1995; Koketsu *et al.*, 2003).

The analysis of Hitchner B1 strain of NDV HN sequence showed that the sialic acid binding analogue to that of the influenza neuraminidase activity protein is the sequence: asn arg lys ser cys ser, between amino acid positions 234 and 239 in NDV HN (Sakaguchi *et al.*, 1989; Gould *et al.*, 2003). This sequence is well conserved among other paramyxoviruses that have been analysed (parainfluenza 3, Sendai virus) and exactly the same amino acids are predicted at the same position in the HN of Beaudette C strain of NDV that have been sequenced (Schaper *et al.*, 1988). The conserved region between NDV and sendai virus is: gly ala glu gly arg leu at amino acid positions 399 to 404 in NDV shows similarity to influenza A sialic acid receptor binding site. This sequence is also found in B1 strain of NDV

(Gould *et al.*, 2003).

Sialic acids act as masks to prevent biological recognition, thus playing the role of maintaining the life span of molecules and cells which they protect (Schauer, 1982; 1985). However, it is known that viruses, bacteria and protozoan parasites recognize sialic acids receptor sites and bind to them on cell surfaces via haemagglutinin, and consequently, exert deleterious effects on their hosts (Traving and Schauer, 1998; Christensen and Bisgaard, 2000).

It has been established that during the life span of red blood cells (RBCs), sialic acids are also removed stepwise from the surface of the cells by action of serum neuraminidases, and by spontaneous chemical hydrolysis (Durocher *et al.*, 1975; Schauer and Kamerling, 1997), thereby exposing the desialylated RBCs to destruction by reticulo-endothelial system.

POSSIBLE ROLE OF NEURAMINIDASE IN PATHOGENICITY OF NEWCASTLE DISEASE

The Paramyxovirus haemagglutininneuraminidase (HN) protein from NDV is a multifunctional protein which is responsible for binding to cellular sialylglycoconjugate receptors, promotion of fusion through interaction with the second viral surface fusion (F) glycoprotein, and processing progeny virons by removal of sialic acid from newly synthesized viral coat protein (Crennell *et al.*, 2000; Connaris *et al.*, 2002). This process of sialic acid removal is vital in the pathogenicity of many diseases, affecting both man and animals.

Neuraminidases are key enzymes of sialic acids catabolism, hydrolyzing the glycosidic linkage between sialic acid molecules, and the penultimate sugar of the carbohydrates chains of oligosaccharide and glycoconjugates (Nagai *et al.*, 1976).

The role of neuraminidases in pathogenesis of disease is controversial. However, certain assumptions have been made. For example, some microbial pathogens' neuraminidases are believed to act as virulence factors, allowing successful competition with the host, by alleviating their spread in host tissue (Godoy *et al.*, 1993). It is also believed that neuraminidases unmask the sub-terminal host cell structures, which then serve as receptors for the parasites and toxins, as in the case of cholera (Gallen *et al.*, 1992). Neuraminidases enable the release of viral progeny by the cleavage of host sialic acid (Wehmann *et al.*, 2003).

The action of neuraminidases on erythrocytes' sialic acids could result in anaemia in animals (Figure 1). This is because it is believed that neuraminidases can remove the sialic acids, which cover the RBCs. As a result, the galactose residues are demasked on the RBCs surfaces, thus presenting a signal for degradation by liver hepatocytes (Durocher *et al.*, 1975; Esievo *et al.*, 1982; Schauer, 1982; Wen *et al.*, 2000).

Chickens inoculated with NDV Kudu 113 strain was observed to develop anaemia which was pronounced during the period of high neuraminidase activity. This was coupled with negative and significant correlations between neuraminidase activity and erythrocytes surface sialic acid concentrations ($r = -0.764$, $P<0.001$), and between neuraminidase activity and packed cell volume (PCV) ($r = -0.792$, $P<0.001$). These results became presumptive evidence of a close relationship between circulating NDV Kudu 113 strain, the production of neuraminidase and accelerated erythrocytes destruction. Therefore, the acute anaemia observed in the infected chickens was attributed to the activities of the circulating NDV Kudu 113 strain, which produced neuraminidase, and in turn cleaved off erythrocytes surface sialic acid from RBCs, thus rendering them more prone to erythrophagocytosis (Oladele *et al.*, 2002b;

Oladele, 2005). This could be responsible for the scanty phenomenon of erythrophagocytosis observed histopathologically in the liver of infected chickens as a result of desialylation of erythrocytes by neuraminidase (Oladele, 2005).

Durocher *et al.* (1975) found that following injection of desialylated ^{51}Cr-labelled erythrocytes into rats and rabbits, there was a rapid clearance of desialylated erythrocytes from circulation, with sequestration in the liver. Kaptzan *et al.* (2000) and Shibuya (2001) also found that reduction in erythrocytes sialic acid contents rendered the RBCs more vulnerable to phagocytosis by macrophages. Also in the mice, it was found that apoptotic cells were recognized and phagocytosed by macrophages, and the molecular property of these cells, recognized by macrophages was the loss of cell surface sialic acids (Itzhaki *et al.*, 2000).

In previous studies by Oladele (2005) the reduced erythrocytes surface sialic acid concentrations observed during the period of acute anaemia probably contributed to the reduction in infected chickens' erythrocytes half-life. Similar assumption was made in bovine trypanosomosis, that significant reduction in erythrocytes surface sialic acid concentrations in infected animals, during the period of anaemia, might be contributing, at least in part (Magaji, 1975; Esievo *et al.*, 1982; Lipkind and Shimanter, 1986), to the reduced erythrocytes half-life observed in trypanosomosis. Also, studies on human erythropoietin have shown that direct relationship exists between sialic acid-containing carbohydrate and its serum half-life (Egrie and Browne, 2001).

Although Cheville and Beard (1972) and Cheville *et al.* (1972) attributed the frequent anaemia in NDV infection to be due, at least in part, to replication of the virus in the host cells and lysis of erythrocytes, the *in vivo* removal of erythrocytes surface sialic acid by NDV Kudu 113 strain neuraminidase which consequently, resulted in erythrophagocytosis by macrophages, has added another mechanism to the pathogenesis of NDV (Oladele, 2005).

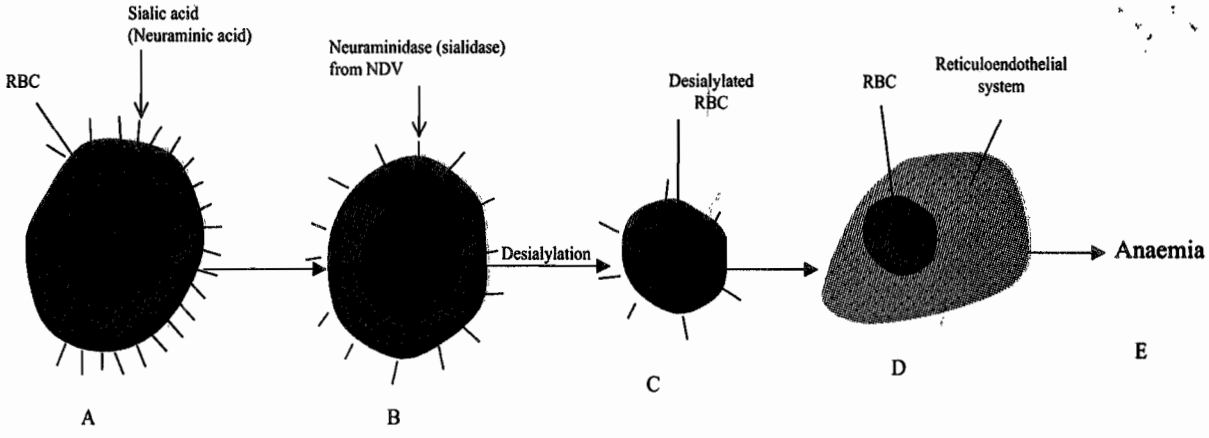

Figure 1:Schematic diagram showing the role of neuraminidase in inducing anaemia in poultry in Newcastle disease

A:Normal RBC masked by sialic acids (Neuraminic acids)
B:NDV neuraminidase attacks the RBC and cleaves off sialic acids
C:Desialylated RBC as a result of cleavage of sialic acids by neuraminidase
D:Desialylated RBC is engulfed by reticuloendothelial system
E:Anaemia ensues as a result of reduced number of circulating RBC

Also in previous studies in infected chickens, NDV Kudu 113 strain induced necrosis and depletion of reticulo-endothelial cells of the spleen, intestine, caecum and other intestinal lymphoid tissues (Oladele, 2005). These findings were in line with the results of Cheville *et al.* (1972), Lam and Hao (1987), Lam and Vasioncelos (1994), and Lam (1996) who found that virulent NDV strains induced the disappearance of lymphoid tissues, necrosis of spleen, vacuolation of lymphoid tissues, destruction of lymphocytes and lymphopaenia. The exact mechanism of lymphoid depletion in NDV infection is still unknown. However, from histopathological findings, Cheville and Beard (1972) postulated that NDV could be lymphocidal. Furthermore, Woodruff and Woodruff (1972) postulated that after NDV infection, the lymphocyte surface receptors could be altered and their migration patterns changed, so as to cause seeding of lymphocytes in the lymphoid organs. It is therefore, reasonable to surmise that the neuraminidase produced by the NDV Kudu 113 strain as reported by Oladele (2005) might have cleaved sialic acid off lymphocytes too, thus altering their surface receptors and migrating patterns.

During the studies of the effects of neuraminidase on RBCs of chickens naturally infected with NDV, the erythrocytes surface sialic acid concentration obtained from chicken naturally infected with NDV was significantly lower ($P<0.001$) than mean values obtained from apparently healthy chickens (Oladele *et al.*, 2002b). This suggests that the reduction in erythrocytes surface sialic acid of chickens that were naturally infected with NDV was probably a mechanism of erythrocytes destruction as previously observed by Durocher *et al.* (1975).

Furthermore, Oladele (2005) found negative and significant correlations between neuraminidase activity and erythrocytes surface sialic acid concentration ($r = -0.447$, $P<0.001$) and between neuraminidase activity and PCV ($r = -0.698$, $P<0.001$) in chickens that were naturally infected with NDV. These results suggest that the presence of NDV might have caused increased neuraminidase activity in circulation, drastic cleavage of erythrocytes surface sialic acids, and hence increased erythrocytic senescence and removal from circulation, with reduction in the PCV value of chickens naturally infected with NDV (Durocher *et al.*, 1975; Oladele *et al.*, 2002b).

It was also observed that chickens vaccinated with NDV Komorov vaccine had higher daily mean values of neuraminidase, free serum sialic acid and haemagglutination inhibition antibodies than their counterparts that were vaccinated with NDV La Sota vaccine (Oladele *et al.*, 2006). This result suggests that the level of neuraminidase and free serum sialic acid concentrations in chickens vaccinated with NDV vaccines will depend among other things, on the pathogenicity and or virulence of the viruses from which the NDV vaccines were produced.

CONCLUSION

The role of neuraminidase in the pathogenicity of ND (in *in vitro* and *in vivo* studies, in naturally occurring NDV infections and in chicken vaccinated with NDV vaccines) has been reviewed. The precise intra and extracellular pathological roles of this enzyme during NDV infection, to some extent, remain obscure. Further elucidations of the role(s) of neuraminidase in the pathogenicity of ND is required for better understanding of the pathogenesis of the disease, and consequently, assist in the management, control and eradication of ND in poultry.

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