HOW INFLUENZA'S NEURAMINIDASE PROMOTES ViroLENCE
AND Creates LOcalized LUNG MUCOSA IMMUNODEFICIENCY

AJAY BHATIA¹ and RICHARD E. KAST²*

¹Ohio State University, Department of Psychiatry, Harding Hospital,
1670 Upham Drive, Columbus OH, 43210 USA, ²University of Vermont,
Department of Psychiatry, 2 Church Street, Burlington, VT 05401 USA

Abstract: Neuraminidase (NA) is an enzyme coded for by the genome of
influenza critical for its pathogenicity and survival. Three currently accepted
roles for this NA in promoting influenza virulence are: 1. NA cleaves newly
formed virus particles from the host cell membrane. Without NA, newly formed
virus would remain attached to the cell within which it was produced. 2. NA
prevents newly released virus particles from aggregating to each other,
preventing clumping that would reduce dissemination. 3. NA promotes viral
penetration of sialic acid-rich mucin that bathes and protects respiratory
epithelium through which the virus must spread and replicate. We outline here
previous research evidence of two further, albeit hypothetical, functions of NA
that together could cause disruption the mucosa-IgA axis, creating localized
partial immunosuppressed state, enhancing both influenza infection itself and
secondary bacterial pneumonia: 4. IgA provides primary immunoglobulin
defense of mucosal surfaces. The hinge region of IgA is normally siaIylated. IgA
denuded of sialic acid is recognized, bound, and cleared by hepatic
asialoglycoprotein receptor (ASGPR). Thus, IgA exposed to free NA would be
so denuded and have increased hepatic clearance. 5. NA removes sialic acid
moieties from mucosa-residing gamma/delta T cells or IgA producing B cells.
Previous work indicates desialylation of these lymphocytes' outer cell membrane
results in altered homing, to bone marrow, away from mucosa. Currently
marketed NA inhibitors oseltamivir (Tamiflu) and zanamivir (Relenza) are FDA
approved in USA for influenza prophylaxis and treatment. These NA inhibitors
lower incidence of secondary bacterial infection in cases where an influenza
infection occurs despite their use. Moreover, they are ameliorative in patients
with secondary bacterial infections treated with antibiotics, a benefit that

* Author for correspondence: email: rekast@email.com

Abbreviations used: ASGPR – asialoglycoprotein receptor; HA – haemagglutinin; IgAN –
IgA nephropathy; NA – neuraminidase; NANA – N-acetyl-neuraminic acid
surpasses the treatment of antibiotics alone. We interpret these last two points as indicating our ascription of localized immunosuppression to influenza's NA could be correct and lead to new treatments of infections generally.

**Key words:** Asialoglycoprotein receptor, IgA, Immunodeficiency, Influenza, Lymphocyte homing, Neuraminidase, Oseltamivir, Sialic acid, Zanamivir

**INTRODUCTION**

This paper reviews three established roles of viral coded neuraminidase (NA) in mediating influenza infection and points out evidence for two further unproven but possible pathogenic functions of this enzyme, not previously described, that lead to a localized lung mucosa immunosuppression. Influenza [recently reviewed in ref. 1] is a single stranded RNA virus, member of the orthomyoviridae family. It remains one of the most significant infectious diseases in the world today with over a billion people infected each year. Moreover, influenza and secondary pneumonia consequent to it represent the sixth leading cause of death in the United States for example, with approximately 36,000 deaths and 114,000 hospitalizations every influenza season in that country alone. It is therefore important to understand the mechanisms of influenza's survival strategies, how it infects cells and how it disrupts host immune responses to itself. Two NA inhibitors: oseltamivir (Tamiflu) and zanamivir (Relenza) are currently marketed to treat and/or to prevent influenza infection. Evidence that they reverse or partially reverse influenza's lung mucosa immunosupression will be presented here. We outline how influenza's NA could hypothetically mediate in a subset of infected individuals, development of IgA nephropathy (IgAN). NA inhibitors would be potentially effective elements of treatment for IgAN should the conjectures of this paper prove to be correct.

**ROLES OF HAEMAGGLUTIN AND NEURAMINIDASE**

On the surface of influenza viruses are two glycoproteins: Haemagglutinin (HA) and NA. Both critical to the spread of the virus. HA initiates virus binding to receptors on the surface of host cells and promotes fusion of virus and host cellular membranes. Viral RNA is thereby able to penetrate the cell. Replication ensues not long thereafter. NA is mechanistically central to influenza's infective potential in several ways. The currently accepted view of the roles of influenza’s NA includes three distinct functions:

1. NA cleaves the cellular-receptor sialic acid residues to which newly formed virions are attached [N-acetyl-neuraminic acid (NANA) and sialic acid are synonyms]. This cleavage releases the newly formed viruses from the cell and allows them to freely invade new cells [1].
2. NA prevents the newly formed virions from binding to one another [3-6]. This ensures that the produced virus does not aggregate. By diffusing widely instead of clumping together, influenza virus maximizes its chance of infecting more cells.

3. Mucin that forms a barrier over epithelial cells of the airway is rich in sialic acid moieties. NA facilitates influenza’s ability to penetrate through this obstacle, thus enhancing infectivity [1-2]. Below is listed enough preliminary research evidence to permit hypothesizing existence of two further functions of NA in influenza, elegantly, both of them would contribute to a lung mucosa localized immunosuppression, should they prove to be true.

4. Because serum protein electrophoresis shows IgG the quantitatively predominant immunoglobulin, it is surprising for clinicians to realize that for the body as a whole more immunoglobulin A (IgA) is synthesized than IgG. Fully 70% of the body’s plasma cells are dedicated exclusively to IgA production [7]. This attests to the importance of mucosal protection and the relative weighting of attack that the body faces. IgA is produced on mucosal surfaces (e.g., gastrointestinal, respiratory, urinary tracts) and is thought to provide a first-line of defense against foreign antigens. IgA’s hinge region covalently binds sialic acid. When IgA’s hinge region is fully sialylated, it circulates freely and is cleared slowly. If exposed to NA, sialic acid will be removed from IgA’s hinge region, consequently disrupting the normally existing homeostasis of IgA in the host. Thus, removal of sialic acid moieties from IgA molecules results in IgA becoming a ligand for the liver’s asialoglycoprotein receptor (ASGPR) [8], increasing IgA clearance from the body. The result would hasten the removal of influenza-directed IgA molecules, weakening the body’s anti-influenza defense. For this mechanism to operate mucosal IgA and circulating IgA would have to equilibrate, data on IgA trafficking that we do not yet have.

5. The mucosa-IgA axis normally results from IgA secreting B-cells that are under the control of gamma-delta T cells. Only 5% of circulating T lymphocytes are gamma-delta T cells. However, they represent up to 15% of small intestine mucosal and up to 40% of large intestine mucosal T-cells [9-11]. If NA removes sialic acid moieties from the surface of either gamma-delta T cells or mucosa-residing B cells, this will disrupt the mucosa-IgA axis. Crucially, desialylation of surface receptors on the outer cell membrane results in shifting lymphocyte homing from mucosa to bone marrow [12-14]. Thus NA may enhance virus survival by tending to depopulate the mucosa of IgA secreting lymphocytes.

Direct experimental evidence showing that influenza’s NA actually does these last two points is missing but there are several parallels with previously recognized examples of IgA desialylation by bacterial NA:

A. Oral viridans type streptococci contribute to human dental plaque. They have significant NA and protease activity that specifically recognizes the
terminal sialic acid of the glycosylated side chain at the IgA hinge region (the NA) and the desialylated IgA hinge region (the protease) [15]. Due to steric hindrance the hinge region is somewhat resistant to proteolytic cleavage, becoming significantly less resistant after desialylation. Importance of hinge region sialic acid for resistance to proteolytic cleavage was first postulated in 1974 [16, 17] being confirmed many times since [15, 18]. Reinholdt et al. suggested in 1990 that oral streptococci use neuraminidase/protease to weaken IgA mediated immunity directed to them. In the current paper we suggest that influenza NA weakens secretory immune responses similarly: NA removes sialic acid from the IgA hinge region, removing the steric hindrance that sialic acid confers, and leaves it denuded. Instead of proteolytic cleavage, influenza relies on hepatic ASGPR recognition of the newly exposed IgA hinge region, and removes it from the circulation. In this way, influenza virus (NA activity and subsequent ASGPR recognition and clearance) parallels streptococci viridans (NA activity and subsequent proteolytic activity).

B. During streptococcal pneumonia, mucosal IgA is seen bound to these bacterias' outer cell membrane [19]. As infection progresses, IgA is seen to be progressively less sialylated [19]. In addition to degradation of IgA integrity, the deisialylation of other defensive proteins as well is believed to enhance this bacteria's survival. Sialic acid's role in protection and maintainance of circulating and mucosal IgA is extensively reviewed in ref. 18.

Our conjecture that influenza's NA alters lymphocyte homing away from mucosa, favoring marrow, has not been well documented but post translation addition to or subtraction of sialic acid on lymphocyte surface glycoproteins has been wellrecognized as one of the central mechanisms directing lymphocyte homing patterns [20].

LOCALIZED IMMUNOSUPPRESSION

Desialylation of IgA [Points 4 above] and NA mediated lymphocyte homing shift away from mucosa [Point 5 above] would result in a localized partial immunosuppressed state in the lung, mitigating the body’s natural system of guarding from infection by interfering with the mucosa-IgA axis. Note the potential synergy between NA-mediated anti-influenza IgA depletion (point 4 above) and NA-mediated mucosal depopulation of IgA-synthesizing lymphocytes (point 5 above). Disrupting production of IgA in this manner increases influenza’s infective and survival potential. It would also leave the body vulnerable to other infective agents: It is understood that a synergism exists between influenza virus and bacterial pathogens [21, 22, 23]. However, mechanisms underlying them remain unclear [24]. Disruption of the host’s IgA-mucosa axis, as outlined here, may account for this synergy. Moreover, it would explain the ameliorative effect NA inhibitors have compared to antibacterial use alone in cases of bacterial pneumonia secondary to an
influenza infection [24, 25]. In addition, NA inhibitors used in the treatment of influenza alone have been shown to reduce the incidence of secondary bacterial pneumonia, and the subsequent need for antibiotics was lowered even when influenza itself was not prevented [26-29]. The work of McCullers et al. [25, 30, 31] show empirical evidence of lung localized partial immunosuppression during influenza infection and that this immunosuppression correlates directly with a given influenza strain's NA activity [25, 30, 31]. Limits of clinical resistance to oseltamivir and zanamivir are likely given the central function of NA to mechanics of influenza replication and dissemination in vivo. Though highly conserved, mutation of the viral NA's active site does occur, preventing the NA inhibitors from binding to it, but significantly reduced virulence is commonly seen to be a clinical concomitant [32, 33]. Altered viral NA has correspondingly reduced ability to remove sialic acid, commonly resulting in reduced viral release from host cells, and ultimately lower infectivity [32, 33]. Historically different strains of influenza express differing amounts of NA, and that strains with higher NA amounts have higher frequency of secondary pneumonia and considerably higher mortality than strains with low amounts of NA activity [30]. Alternatively, resistance to NA inhibitors may occur by another route: The HA molecule may mutate becoming less adherent to sialic acid residues, reducing the dependency of the virus on NA to release it from the host's cells. However, the reduced affinity of HA for sialic acid results in a virus that also can no longer bind to host cells as readily, undermining its capacity to initiate infection. NA inhibitors, like many great drugs, act through multiple interrelated mechanisms that enhance their efficaciousness.

WIDER IMPLICATIONS

IgAN is the most common primary cause of glomerulonephritis in the world today. Classically, it presents with fatigue, rising creatinine, microscopic hematuria, and a history of upper respiratory infection (URI) [34]. Although certainly unproven, we conjecture that the URI is a result of influenza or other virus whose NA hastens the removal of IgA by ASGPR but in the process expose excess amounts of IgA to beta-galactosidase. IgA's hinge sialic acid moieties are attached to the IgA via the intermediary beta-galactose. IgA minus sialic acid moieties and then minus its beta-galactose is not recognized by hepatic ASGPR, resulting in formation of circulating immune complexes. Excess IgA ultimately deposits in/on glomerular mesangial cells in the kidney causing inflammation, scarring, and decreased renal function. IgA nephropathy (IgAN) in general, and certain histological subtypes in particular show abnormally increased desialylated IgA species in circulation, believed to be of pathological significance to its glomerular deposition [35]. Specifically, the spectrum of IgA nephropathy biopsies range from mild or no visible changes to
extensive inflammation, mesangial expansion, and glomerularsclerosis. And the degree of pathological abnormalities seen is proportional to desialylated IgA, implicating the degree of desialylation of IgA to inflammation and glomerular injury.

Viral NA may also cause a weighting shift of production of IgA from mucosa to bone marrow prominence by removing sialic acid moieties from mucosa-residing gamma/delta T cells or from B cells that result in their homing to the bone marrow [11-13]. By treating IgAN with NA inhibitors as has been suggested [36], it may be possible to prevent the abnormal IgA (denuded of sialic acid and beta-galactosidase) from ever forming in the first place. Theoretically, this would arrest the illness before any deposition in the mesangium ever occurred. And for the millions worldwide who suffer from IgAN, NA inhibitors with its high threshold of tolerability and low side-effect profile, could represent an effective treatment, even without antecedent or concurrent influenza.

CONCLUSIONS

Intact functional NA is critical to the infectivity of influenza. The currently accepted roles for NA include: 1. mediating release from host cells, 2. penetration of virus particles through sialic acid-rich mucin bathing epithelial linings of the respiratory tract, and 3. preventing newly-formed virus particles from clumping, enhancing dispersal. We recount in this paper preliminary evidence that two additional functions for NA exist: 4. enhancing the removal of IgA by the hepatic ASGPR, and 5. depopulating the mucosa of either residing gamma/delta T cells or IgA producing B cells. These actions would create a local immunosuppressed state in the lung and might explain the high incidence of secondary bacterial pneumonia, common in influenza infections. Moreover, this would be consistent with: 1. the reduced incidence of secondary bacterial infections when influenza is treated with NA inhibitors alone and 2. the ameliorative effect seen when NA inhibitors are used in addition to antibiotics in cases of bacterial pneumonia secondary to influenza infection, a benefit that surpasses that of antibiotic use alone. NA may also be a critical part of the pathogenesis of IgAN, and NA inhibitors may represent a safe, and potentially efficacious treatment.

REFERENCES

1. Moscona, A. Neuraminidase inhibitors for influenza. N. Engl. J. Med. 353 (2005) 1363-1367.
2. Matrosovich, M.N., Matrosovitch, T.Y., Gray, T, Roberts, N.A. and Klenk, H.D. Neuraminidase is important for the initiation of influenza virus infection in human airway epithelium. J. Virol. 78 (2004) 12665-12667.
3. Gubareva, L.V., Kaiser, L. and Hayden, F.G. Influenza virus neuraminidase inhibitors. Lancet 355 (2000) 827-835.
4. Englund, J.A. Antiviral therapy of influenza. Sem. in Ped. Infect. Dis. 13 (2002) 120-128.
5. Stiver, G. The treatment of influenza with antiviral drugs. CMAJ 168 (2003) 49-57.
6. Colman, P.M. A novel approach to antiviral therapy for influenza. J. Antimicrob. Chemother. 44 (1999) 17-22.
7. Brandtzaeg, P., Farstad, I.N., Johansen, F.E., Morton, H.C., Norderhaug, I.N. and Yamanaka, T. The B-cell system of human mucosae and exocrine glands. Immunol. Rev. 171 (1999) 45-87.
8. Tomana, M., Kulhavy, R. and Mestecky, J. Receptor-mediated binding and uptake of IgA by human liver. Gastroenterology 94 (1988) 762-770.
9. Groh, V., Porcelli, S., Fabbi, M., Lanier, L.L., Picker, L.J., Anderson, T., Warnke, R.A., Bhan, A.K., Strominger, J.L. and Brenner, M.B. Human lymphocytes bearing T cell receptor gamma/delta are phenotypically diverse and evenly distributed throughout the lymphoid system. J. Exp. Med. 169 (1989) 1277-1294.
10. Spencer, J., Isaacson, P.G., Diss, T.C. and MacDonald, T.T. Expression of disulfide-linked and non-disulfide-linked forms of the T cell receptor gamma/delta heterodimer in human intestinal intraepithelial lymphocytes. Eur. J. Immunol. 19 (1989) 1335-1338.
11. Deusch, K., Luling, F., Reich, K., Classen, M., Wagner, H. and Pfeffer, K.A major fraction of human intraepithelial lymphocytes simultaneously expresses the gamma/delta T cell receptor, the CD8 accessory molecule and preferentially uses the V delta1 gene segment. Eur. J. Immunol. 21 (1991) 1053-1059.
12. Jones, W.M., Walcheck, B. and Jutila, M.A. Generation of a new gamma/delta T cell-specific monoclonal antibody (GD3.5). J. Immunol. 156 (1996) 3772-3779.
13. Floyd, H., Nitschke, L. and Crocker, P.R. A novel subset of murine B cells that expresses unmasked forms of CD22 is enriched in the bone marrow: Implications for B-cell homing to the bone marrow. Immunology 101 (2000) 342-347.
14. Nitschke, L., Floyd, H. and Ferguson, D.J., Identification of CD22 ligands on CD22 bone marrow sinusoidal endothelium implicated in CD22-dependent homing of recirculating B-cells. J. Exp. Med. 189 (1999) 1513-1518.
15. Reinholdt, J., Tomana, M. and Mortensen, S.B. Molecular aspects of IgA degredation by oral streptococci. Infect. Immunol. 58 (1990) 1186-1194.
16. Kast R.E. A theory of lymphocyte blast transformation and malignant change based on proteolytic cleavage of the trigger peptide: The detendomer. Oncology 29 (1974) 249-264.
17. Kast, R.E. Lymphocytes and cells in malignant transformation. Oncology 32 (1975) 175-189.
18. Gronbaek Frandsen, E.V. Bacterial degradation of IgA1 in relation to periodontal disease. *APMIS* (Suppl) 87 (1999) 1-54.
19. King, S.J., Hippe, K.R., Gould, J.M., Bae, D., Peterson, S., Cline, R.T., Fasching, C., Janoff, E.N. and Weiser, J.N. Phase variable desialylation of host proteins that bind to Streptococcus pneumoniae in vivo and protect the airway. *Mol. Microbiol.* 54 (2004) 159-171.
20. Kannagi, R. Regulatory roles of carbohydrate ligands for selectins in the homing of lymphocytes. *Curr. Opin. Struct. Biol.* 12 (2002) 599-608.
21. Glezen, W.P., Payne, A.A. and Snyder, D.N. Mortality and influenza. *J. Infect. Dis.* 146 (1982) 313-321.
22. Simonsen, L, Fukada, K. and Schonberger, L.B. The impact of influenza epidemics on hospitalizations. *J. Infect. Dis.* 181 (2000) 831-837.
23. Simonsen, L. The global impact of influenza on morbidity and mortality. *Vaccine* 17 (Suppl 1) (1999) S3-10.
24. McCullers, J.A. and Bartmess, K.C. Role of neuraminidase in lethal synergism between influenza virus and streptococcus pneumoniae. *J. Infect. Dis.* 187 (2003) 1000-1009.
25. McCullers, J.A. Effect of antiviral treatment on the outcome of secondary bacterial pneumonia after influenza. *J. Infect. Dis.* 190 (2004) 519-526.
26. Kaiser, L., Wat, C., Mills, T., Mahoney, P., Ward, P. and Hayden, F. Impact of oseltamivir treatment on influenza-related lower respiratory tract complications and hospitalizations. *Arch. Intern. Med.* 163 (2003) 1667-1672.
27. Kaiser, L., Keene, O.N. and Hammond, J.M. Impact of zanamivir on antibiotic use for respiratory events following acute influenza in adolescents and adults. *Arch Intern Med* 160 (2000) 3234-3240.
28. Treanor, J.J., Hayden, F.G., Vroooman, P.S., Barbarash, R., Bettis, R., Riff, D., Singh, S., Kinnersley, N., Ward, P. and Mills, R.G. Efficacy and safety of oral neuraminidase Inhibitor oseltamivir in treating acute influenza: A randomized controlled trial. *JAMA* 283 (2000) 1016-1024.
29. Monto, A.S., Webster, A. and Keene, O. Randomized, placebo-controlled studies of inhaled zanamivir in the treatment of influenza A and B: Pooled efficacy analysis. *J. Antimicrob. Chemother.* 44 (1999) 23-29.
30. Peltola, V.T., Murti, K.G. and McCullers, J.A. Influenza virus neuraminidase contributes to secondary bacterial pneumonia. *J Infect Dis.* 192 (2005) 249-257.
31. Peltola, V.T. and McCullers, J.A. Respiratory viruses predisposing to bacterial infections: role of neuraminidase. *Pediatr. Infect. Dis. J.* 23 (Suppl.1) (2004) S87-97.
32. Yen, H.L., Herlocher, L.M., Hoffmann, E., Matrosovich, M.N., Monto, A.S., Webster, R.G. and Govorkova, E.A. Neuraminidase inhibitor-resistant influenza viruses may differ substantially in fitness and transmissibility. *Antimicrob. Agents Chemother.* 49 (2005) 4075-4084.
33. Roberts, N. Treatment of influenza with neuraminidase inhibitors: Virological implications. *Phil. Trans. R. Soc. Lond.* **356** (2001) 1895-1897.

34. Wakai, K., Nakai, S., Matsuo, S., Kawamura, T., Hotta, N., Maeda, K. and Ohno, Y. Risk factors for IgA nephropathy: A case-control study with incident cases in Japan. *Nephron* **90** (2002) 16-23.

35. Xu, L.X. and Zhao, M.H. Aberrantly glycosylated serum IgA1 are closely associated with pathologic phenotypes of IgA nephropathy. *Kidney Int.* **68** (2005) 167-172.

36. Altschuler, E.L., Bhatia, A. and Kast, R.E. Consideration of use of neuraminidase inhibitors such as oseltamivir and zanamivir in IgA nephropathy. *Kidney Int.* **68** (2005) 2910-2911.