Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Virus Research, 25 (1992) 145–153

VIRUS 00810

Increased influenza A virus sialidase activity with *N*-acetyl-9-*O*-acetylneuraminic acid-containing substrates resulting from influenza C virus *O*-acetylesterase action *

I. Muñoz-Barroso [a], A. García-Sastre [a], E. Villar [a], J.-C. Manuguerra [b], C. Hannoun [b] and J.A. Cabezas [a]

[a] *Departamento de Bioquímica y Biología Molecular, Facultad de Biología, Universidad de Salamanca, Salamanca, Spain, and* [b] *Unité d'Ecologie Virale, Institut Pasteur, Paris, France*

(Received 25 February 1992; revision received and accepted 15 May 1992)

Summary

Influenza virus type C (Johannesburg/1/66) was used as a source for the enzyme *O*-acetylesterase (EC 3.1.1.53) with several natural sialoglycoconjugates as substrates. The resulting products were immediately employed as substrates using influenza virus type A [(Singapore/6/86) (H1N1) or Shanghai/11/87 (H3N2)] as a source for sialidase (neuraminidase, EC 3.2.1.18). A significant increase in the percentage of sialic acid released was found when the *O*-acetyl group was cleaved by *O*-acetylesterase activity from certain substrates (bovine submandibular gland mucin, rat serum glycoproteins, human saliva glycoproteins, mouse erythrocyte stroma, chick embryonic brain gangliosides and bovine brain gangliosides). A common feature of all these substrates is that they contain *N*-acetyl-9-*O*-acetylneuraminic acid residues. By contrast, no significant increase in the release of sialic acid was detected when certain other substrates could not be de-*O*-acetylated by the action of influenza C esterase, either because they lacked *O*-acetylsialic acid (human glycophorin A, α_1-acid glycoprotein from human serum, fetuin and porcine submandibular gland mucin) or because the 4-*O*-acetyl group

Correspondence to: J.A. Cabezas, Departamento de Bioquímica y Biología Molecular, Facultad de Biología, Universidad de Salamanca, Plaza de la Merced 1, 37008 Salamanca, Spain. Fax: (34) (23) 294513.

* Part of the results of this paper was presented at the 8th Workshop of the European Study Group on Lysosomal Diseases held in October, 1991, in Annecy, France.

was scarcely cleaved by the viral *O*-acetylesterase (equine submandibular gland mucin). The biological significance of these facts is discussed, relative to the infective capacity of influenza C virus.

Influenza C virus; *O*-Acetylesterase; Sialidase; Neuraminidase; Glycoconjugates

---

O-Acetylesterase (EC 3.1.1.53, *N*-acyl-*O*-acetylneuraminate *O*-acetylhydrolase) and sialidase (EC 3.2.1.18, neuraminidase, acylneuraminyl hydrolase, exo-α-sialidase) (Cabezas, 1991) are surface components of influenza viruses which act as receptor-destroying enzymes (RDE). This sialidase is found in influenza A and B viruses, but not in influenza C virus. In contrast, *O*-acetylesterase is found in influenza C virus (but not in influenza A and B viruses) (Herrler et al., 1979; Herrler et al., 1981) as a component of the HEF glycoprotein, the only myxovirus trifunctional protein showing hemagglutinin (H) and esterase (E) activities and fusion power (F) (Herrler et al., 1988a; Herrler et al., 1988b). Furthermore, a hemagglutinin-esterase (HE) has been recently found in coronaviruses. Knowledge of the composition and functional differences between influenza viruses A or B and C is now considered sufficient to include influenza virus type C as a new genus in the future classification of virus (Cabezas et al., 1991).

The role of both *O*-acetylesterase and sialidase in the biological cycle of influenza viruses is considered to be very important in the propagation of the virus (since both enzymes could contribute to avoiding the aggregation of the virions on the host cell), although the action mechanisms are not yet well understood. However, Roger et al. (1986) and Herrler and Klenk (1987) have found that influenza C virus uses *N*-acetyl-9-*O*-acetylneuraminic acid as a high affinity receptor determinant for attachment to cells.

Since, (1) some outbreaks of influenza produced by influenza C virus can occur in coincidence with outbreaks produced by influenza B virus at least (Gerber et al., 1952), (2) infection by influenza C virus occurs in nearly all age groups including children (Gerber et al., 1952; Katagiri et al., 1983; Katagiri et al., 1987; Manuguerra et al., 1991b) and elderly persons (Homma et al., 1982; Nishimura et al., 1987; O'Callaghan et al., 1980; O'Callaghan et al., 1983), even among those living in isolated communities (Nishimura et al., 1987), and (3) recurrent infection with influenza C virus occurs frequently (Katagiri, 1983) and in certain cases with severity (Dykes et al., 1980), it seemed interesting to investigate whether the action of the viral *O*-acetylesterase on some natural substrates could affect the action of the viral sialidase.

The virus strains used were A/Singapore/6/86 (H1N1), A/Shanghai/11/87 (H3N2) and C/Johannesburg/1/66 (briefly designated as C/JHB/1/66). The viruses were grown and purified as previously reported (Cabezas et al., 1982; García-Sastre et al., 1991; Manuguerra et al., 1991a). The high degree of purity of the final influenza virus preparations was checked by SDS/PAGE electrophoresis (Laemmli, 1970; García-Sastre et al., 1991).

Mouse erythrocyte stroma was prepared in our laboratories after hemolysis of 3-month-old Balb/c mouse red cells (Manuguerra et al., 1991a). Rat serum glycoproteins were prepared by precipitation with ethanol and lyophylization of rat serum. Human saliva glycoproteins were obtained as previously reported (Cabezas et al., 1964). Gangliosides from 20-day chick embryonic brains were prepared by Dr. C. Dubois (Paris), as previously described (Dubois et al., 1990). In this procedure, attention was paid to avoiding alkaline conditions which could induce de-*O*-acetylation of the gangliosides. Equine submandibular gland mucin was a gift from Dr. R. Pfeil (Kiel, Germany) and porcine submandibular gland mucin was a gift from Dr. L. Warren (Philadelphia, USA). All other reagents were obtained from Sigma (St. Louis, MO, USA) and were of the highest purity commercially available.

Sialidase activity was determined in influenza A virus samples by a fluorimetric procedure (Warner and O'Brien, 1979), essentially as previously described (Cabezas et al., 1982; Cabezas et al., 1983), measuring the relative fluorescence of the 4-methylumbelliferone released by the sialidase activity from the substrate 4-methylumbelliferyl-*N*-acetylneuraminic acid after 10 min of incubation at 37°C in 200 mM potassium phosphate buffer, pH 6.8. *O*-Acetylesterase activity in influenza C virus samples was determined using 4-methylumbelliferyl-acetate as substrate, as previously reported (García-Sastre et al., 1991; Schauer et al., 1988a). When glycoproteins and gangliosides were used as substrates, the acetate released was measured with a commercial test kit from Boehringer (Mannheim, Germany; cat. no. 148 261) at 340 nm in a Cary 219 or in a Hitachi U-2000 spectrophotometer. Total sialic acids (expressed as *N*-acetylneuraminic acid) were determined by the modified (Miettinen and Takki-Luukkainen, 1959) resorcinol procedure (Svennerholm, 1957). Free sialic acid was determined by the periodate-thiobarbiturate method (Aminoff, 1961; Warren, 1959).

Assays to measure both sialate *O*-acetylesterase and sialidase activities were carried out at 37°C for 1 h in incubation mixtures containing 5 μl (240 mU) of influenza C/JHB/1/66 virus in 20 mM potassium phosphate buffer (pH 7.6) and the substrate (about 500 μg glycoprotein, with 7–50 μg total sialic acid). The reaction was stopped by freezing. Then, 5 μl (252 mU) of influenza A virus preparation was added in 200 mM potassium phosphate buffer (pH 6.2). Incubation was carried out at 37°C for 1 h and stopped by freezing. Influenza C virus, inactivated at 100°C for 15 min, was used as a blank. Parallel assays for measuring the sialidase activity of influenza A virus were also run, containing a blank of influenza C virus inactivated at 100°C for 15 min. Another blank was included, which contained inactivated influenza C virus at 100°C plus influenza A virus inactivated at 100°C.

When a mixture of bovine brain gangliosides was used as substrate, the assays were carried out with samples of 175 μg (containing 50 μg total sialic acid, as *N*-acetylneuraminic acid). In some assays, gangliosides were previously de-*O*-acetylated by adding an equal volume of ammonium hydroxide (concentrated) to the sample. Then, incubations for 2 h at room temperature and evaporation over N_2 for 72 h were carried out, as previously described (Ravindranaths et al., 1988).

Protein concentrations were determined (Lowry et al., 1951) using bovine serum albumin as standard. One unit of enzyme (U) was defined as the amount of enzyme which releases 1 μmol of product (4-methylumbelliferone or acetate) per minute under the assay conditions.

Table 1 shows that the percentage of sialic acid released by influenza A virus sialidase with *N*-acetyl-9-*O*-acetylneuraminic acid-containing substrates [bovine submandibular gland mucin (BSM), rat serum glycoproteins, human saliva glycoprotein and mouse erythrocyte stroma glycoconjugates] was significantly increased when these compounds had previously been subjected to the action of influenza C virus *O*-acetylesterase.

However, no significant differences were found in the percentage of sialic acid(s) released from substrates lacking *O*-acetyl groups (human glycophorin A, α_1-acid glycoprotein from human serum, fetuin and porcine submandibular gland mucin) treated by *O*-acetylesterase plus sialidase and the respective substrates subjected to the action of sialidase alone (*O*-acetylesterase was previously inactivated by heating).

Although equine submandibular gland mucin contains an *O*-acetylsialic acid, the viral *O*-acetylesterase only works at a very slow rate. This sialic acid is *N*-acetyl-4-*O*-acetylneuraminic acid, with substitution at the hydroxyl group on carbon 4 (Fig. 1, II).

Human glycophorin A has not been reported to contain Neu5,9Ac$_2$ but does contain *N*-acetylneuraminic acid (Blumenfeld and Adamany, 1978; Gahmberg et al., 1983). As previously reported by us (García-Sastre et al., 1991), we have confirmed that glycophorin was not hydrolysed by the viral *O*-acetylesterase. However, this result is not in agreement with the suggestion of Nishimura et al. (1988) 'that most influenza C virus receptors on human erythrocytes, if not all, reside on glycophorin A'. In contrast, mouse erythrocyte stroma glycoconjugates, which contain Neu5,9Ac$_2$ (Reuter et al., 1991), were cleaved by the viral *O*-acetylesterase.

We also found that acetate was released from BSM, rat serum glycoproteins and human saliva glycoproteins by the action of influenza C virus *O*-acetylesterase, as expected, whereas human glycophorin A, α_1-acid glycoprotein from human serum, fetuin and porcine submandibular gland mucin were not cleaved (Table 1).

Our results on the hydrolysis of chick embryonic and bovine brain gangliosides, which contain Neu5,9Ac$_2$ (Dubois et al., 1990; Zimmer et al., 1991), disclosed a low percentage of sialic acid released after treatment by esterase plus sialidase when the assays were carried out without a detergent (Table 1). Similar results were obtained when bovine brain gangliosides were de-*O*-acetylated with ammonium hydroxide. The low degree of hydrolysis after both types of treatment could be due to the experimental conditions, which probably were not similar to those of the cell. Therefore, we added deoxycholate, as a detergent. This led to a remarkable increase in the percentage of sialic acid released (Table 1). This increase was higher when influenza C virus *O*-acetylesterase acted prior to influenza A virus sialidase on either chick embryonic or bovine brain gangliosides.

TABLE 1

Activity of *O*-acetylesterase (influenza C/JHB/1/66 virus) and sialidase (influenza A/Singapore/6/86) towards several natural substrates

Substrate	Type of sialic acid (%)					Sialic acid released (%) after treatment by		Acetate released (μg acetate/mg total sialic acid) after treatment by influenza C virus (*O*-acetylesterase)
	N,O, Diacetylneuraminic acids		Other sialic acids					
	Neu5,9Ac$_2$	Neu4,5Ac$_2$	Neu5Ac	Neu5Gc	Lactyl-NeuAc	Influenza C virus (*O*-acetylesterase) [Inactivated by heat] + Influenza A virus (sialidase)	Influenza C virus (*O*-acetylesterase) + Influenza A virus (sialidase)	
Bovine sub. mucin (BSM) [a]	≈ 50		+	≈ 40		3.2 ± 0.4	8.8 ± 0.1	23
Rat serum glycoproteins [b]	+ +					13.8 ± 0.3	34.0 ± 0.2	84
Human saliva glycoproteins [c]	+		+ +		+	0.7 ± 0.5	4.3 ± 0.5	22
Mouse erythrocyte stroma [d]	+ +		+	+		2.7 ± 0.3	4.5 ± 0.3	N.D.
Chick embryonic brain gangliosides [e]	+		+					
without deoxycholate						0.0 + 0.2	0.0 + 0.1	N.D.
with deoxycholate *						16.5 ± 0.1	18.9 ± 0.4	N.D.
Bovine brain gangliosides [f]	+		+					
without deoxycholate						0.0 + 0.2	0.7 ± 0.1	N.D.
with deoxycholate *						16.6 ± 0.1	17.9 ± 0.1	N.D.
Equine sub. mucin [g]		≈ 50	+	+	+	9.9 ± 0.9	10.4 ± 0.5	0
Human glycophorin A [h]			≈ 100			33.3 ± 2.9	34.1 ± 1.7	0
α_1-Acid glycoprotein [i] (from human serum)			≈ 100			20.4 ± 0.4	19.5 ± 0.4	0
Fetuin [j]			≈ 93	≈ 7		21.8 ± 0.2	21.6 ± 0.4	0
Porcine sub. mucin [k]			≈ 10	≈ 90		**	**	0

The values for sialic acid release are the mean of 5 determinations ± S.D., except for gangliosides and glycophorin which were 10 determinations. Those of acetate were obtained in one assay. N.D., not determined. Abbreviations used: Neu5,9Ac$_2$, *N*-acetyl-9-*O*-acetylneuraminic acid; Neu4,5Ac$_2$, *N*-acetyl-4-*O*-acetylneuraminic acid; Neu5Ac, *N*-acetylneuraminic acid; Neu5Gc, *N*-Glycolylneuraminic acid; LactylNeuAc, *N*-Acetyl-9-*O*-L-lactylneuraminic acid. Composition as deduced from the following references: [a] Ref. (Gottschalk and Bhargava, 1972; Schauer et al., 1988a); [b] Ref. (Zimmer et al., 1991); [c] Ref. (Cabezas et al., 1964; Schauer, 1982); [d] Ref. (Reuter et al., 1991); [e] Ref. (Dubois et al., 1990); [f] Ref. (Zimmer et al., 1991); [g] Ref. (Schauer et al., 1988a; Zimmer et al., 1991); [h] Ref. (Blumenfeld and Adamany, 1978; Cabezas et al., 1982; Gahmberg et al., 1983); [i] Ref. (Jeanloz, 1972; Zimmer et al., 1991); [j] Ref. (Faillard and Cabezas, 1963; Zimmer et al., 1991); [k] Ref. (Gottschalk and Bhargave, 1972; Zimmer et al., 1991). * Sodium deoxycholate was used at 5% of the mixture (w/v). ** No typical color.

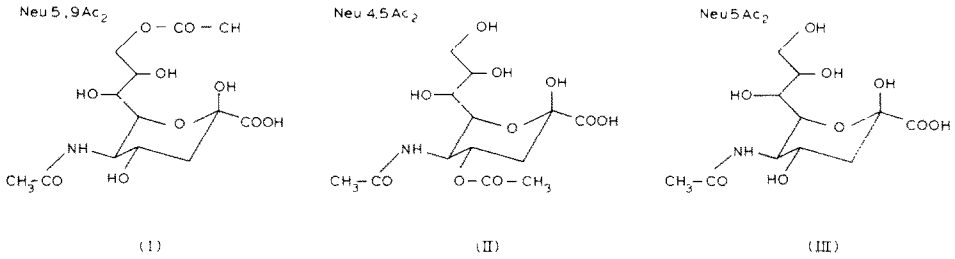

Fig. 1. Structure of *N*-acetyl-9-*O*-acetylneuraminic acid (I), *N*-acetyl-4-*O*-acetylneuraminic acid (II) and *N*-acetylneuraminic acid (III).

Upon treatment of bovine brain gangliosides by ammonium hydroxide for 2 h, a partial de-*O*-acetylation was accomplished and some unidentified degradation products were also originated. Thus, de-*O*-acetylation performed by the viral esterase seems to be more efficient and milder than that due to alkali treatment, at least on bovine brain gangliosides.

The sialidase activity of influenza virus A/Singapore/6/86 (H1N1) was always higher than that of influenza virus A/Shanghai/11/87 (H3N2). Accordingly, we used the former with all substrates here indicated. However, the latter also acted when BSM was used as substrate, although with less efficiency (about 16% lower).

The specificity of influenza C virus *O*-acetylesterase seems to be a complicated matter. In fact, this specificity is very strict towards sialic acids (Schauer et al., 1988a; Schauer et al., 1988b), while it is broad towards very different *O*-acetyl-containing compounds both synthetic and natural non-sialic acid-containing substrates (García-Sastre et al., 1991). However, the very low capacity of influenza C virus *O*-acetylesterase to release the 4-*O*-acetyl group from *N*-acetyl-4-*O*-acetylneuraminic acid, as well as its inactivity towards *N*-acetyl-7-*O*-acetylneuraminic acid (Schauer et al., 1988a), could be explained by the relatively hidden position of both 4-*O*-acetyl (Fig. 1, II) and 7-*O*-acetyl groups in the molecule, and by the possibility of steric hindrance due to the 5-*N*-acetyl group situated in their proximity (Fig. 1). By contrast, the easy accessibility of the 9-*O*-acetyl group because of its terminal position in that physiological substrate of the enzyme which is the Neu5,9Ac_2 (Fig. 1, I) could be similar to that of the *O*-acetyl group(s) in very different *O*-acetyl-containing compounds which act as substrates for this enzyme (García-Sastre et al., 1991).

The possibility that *O*-acetylesterase of influenza virus type C might facilitate or enable the action of the influenza viral sialidase has only been suggested (Schauer and Reuter, 1988; Schauer et al., 1988b), as has the possibility that it might play some role in the entry of the virus into host cells (Vlasak et al., 1989). Our present results demonstrate the possibility of co-operation of influenza C virus (through the action of its *O*-acetylesterase) in the activity of influenza A virus, increasing the effect of influenza A virus sialidase on natural Neu5,9Ac_2-containing substrates. This phenomenon could probably be explained by the better accessibility of

N-acetylneuraminic acid (Fig. 1, III) than that of Neu5,9Ac$_2$ (Fig. 1, I), the former compound working better as substrate for influenza A virus sialidase than the latter.

Finally, the recurrence of infection may play an important role in the persistence of type C influenza virus in humans (Katagiri et al., 1983). In this sense, it is noteworthy that infections produced experimentally in dogs (Ohwada et al., 1986) and naturally in children (Katagiri et al., 1983; Katagiri et al., 1987) by influenza C virus both are accompanied by the presence and shedding of the infective virus in the respiratory tract for a relatively long period of time, in some cases as long as three weeks (Katagiri et al., 1983; Katagiri et al., 1987). Since it is known that sialidase contributes to the propagation of influenza viruses types A and B, it might be suggested that the biological significance of influenza C virus (through its *O*-acetylesterase activity), in co-operation with influenza A or B viruses, could be more important than generally accepted until now. Acetylesterase might contribute to unmasking new receptor sites and to affording enough time for an easier superinfection by type A or B influenza virus.

Acknowledgements

This work was supported by a grant to J.A.C. from FISss (91/00420201E), Spain, which is related to a collaborative research carried out within the framework of the Commission of the European Communities Concerted Action on Ageing and Diseases (EURAGE). We thank Drs. C. Dubois, A. Pfeil and L. Warren for the gift of some samples of substrates, Mrs. M.J. Ruano for her collaboration in the bovine ganglioside typification, N. Skinner for correcting the manuscript and Mr. F.J. Criado and Mrs. E. Albillo for secretarial work.

References

Aminoff, D., (1961) Methods for the quantitative estimation of *N*-acetylneuraminic acid and their application to hydrolysates of sialomucoids. Biochem. J. 81, 384–392.

Blumenfeld, O.O. and Adamany, A. (1978) Structural polymorphism within the amino-terminal region of MM, NN, and MN glycoproteins (glycophorins) of the human erythrocyte membrane. Proc. Natl. Acad. Sci. USA 75, 2727–2731.

Cabezas, J.A. (1991) Some questions and suggestions on the type references of the official nomenclature (IUB) for sialidase(s) and endosialidase. Biochem. J. 278, 311–312.

Cabezas, J.A., Vázquez-Porto, J., Frois, M^a. D., Marino, C. and Arzúa, J. (1964) Acidos siálicos. III. Contenido en ácido siálico y en osas de la saliva humana. Rev. Esp. Fisiol. 20, 77–82.

Cabezas, J.A., Calvo, P., Eid, P., Martín, J., Perez, N., Reglero, A., Rodrigo, M. and Hannoun, C. (1982) Studies on neuraminidase from influenza virus A(H3N2) obtained by two procedures. Int. J. Biochem. 14, 311–319.

Cabezas, J.A., Reglero, A. and Hannoun, C. (1983) A fluorometric procedure for measuring the neuraminidase activity: its application to the determination of this activity in influenza and parainfluenza viruses. Anal. Biochem. 131, 121–126.

Cabezas, J.A., Villar, E., García-Sastre, A., Manuguerra, J.C. and Hannoun, C. (1991) New data on influenza virus type C confirm its peculiarities as a new genus. Intervirology 32, 325–326.

Dubois, C., Manuguerra, J.C., Hauttecoeur, B. and Maze, J. (1990) Monoclonal antibody A2B5, which detects cell surface antigens, binds to ganglioside G_{T3} (II^3 $(NeuAc)_3LacCer$) and to its 9-*O*-acetylated derivate. J. Biol. Chem. 265, 2797–2803.

Dykes, A.C., Cherry, J.D. and Nolan, C.E. (1980) A clinical, epidemiologic, serologic, and virologic study of influenza C virus infection. Arch. Intern. Med. 140, 1295–1298.

Faillard, H. and Cabezas, J.A. (1963) Isolieurung von *N*-acetyl- und *N*-Glykolyl-neuraminsäure aus Kälber und Hühnerserum. Hoppe-Seyler's Z. Physiol. Chem. 333, 266–271.

Gahmberg, C.G. Jokinen, M., Karhi, K.K., Kämpe, O., Peterson, P.A. and Anderson, L.C. (1983) Glycophorin A: in vitro biogenesis and processing. Meth. Enzymol. 96, 281–296.

García-Sastre, A., Villar, E., Manuguerra, J.C., Hannoun, C. and Cabezas, J.A. (1991) Activity of influenza C virus *O*-acetylesterase with *O*-acetyl-containing compounds. Biochem. J. 273, 435–441.

Gerber, P., Woolridge, R.L., Seal, J.R. and Ziegra, S.R. (1952) Epidemic influenza B and C in Navy recruits during winter of 1951–52. Proc. Soc. Expo. Biol. Med. 81, 624–628.

Gottschalk, A. and Bhargava, A.S. (1972) Submaxillary gland glycoproteins. In: A. Gottschalk (Ed.), Glycoproteins. Vol. B. pp. 810–829. Elsevier, Amsterdam.

Herrler, G. and Klenk, H.-D. (1987) The surface receptor is a major determinant of the all tropism of influenza C virus. Virology 159, 102–108.

Herrler, G., Compans, R.W. and Meier-Ewert, H. (1979) A precursor glycoprotein in influenza C virus. Virology 99, 49–56.

Herrler, G., Nagele, A., Meier-Ewert, H., Bhown, A.S. and Compans, R.W. (1981) Isolation and structural analysis of influenza C virion glycoproteins. Virology 113, 439–451.

Herrler, G., Dürkop, I., Becht, H. and Klenk, H.-D. (1988a) The glycoprotein of influenza C virus is the haemagglutinin, esterase and fusion factor. J. Gen. Virol. 69, 839–846.

Herrler, G., Multhaup, Beyreuther, K. and Klenk, H.-D. (1988b) Serine 71 of the glycoprotein HEF is located at the active site of the acetylesterase of influenza C virus. Arch. Virol. 102, 269–274.

Homma, M., Ohyama, S. and Katagiri, S. (1982) Age distribution of the antibody to type C influenza virus. Microbiol. Immunol. 26, 639–642.

Jeanloz, A. (1972) α_1-Acid glycoprotein. In: A. Gottschalk (Ed.), Glycoproteins. Vol. A. pp. 565–581. Elsevier, Amsterdam.

Katagiri, S., Ohizumi, A. and Homma, M. (1983) An outbreak of type C influenza in a children's home. J. Infect. Dis. 148, 51–56.

Katagiri, S., Ohizumi, A., Ohyama, S. and Homma M. (1987) Follow-up study of type C influenza outbreak in a children home. Microbiol. Immunol. 31, 337–343.

Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680–685.

Lowry, O.H., Rosebrough, N.F., Farr, A.L. and Randall, R.J. (1951) Protein measurements with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.

Manuguerra, J.C., Dubois, C. and Hannoun, C. (1991a) Analytical detection of 9(4)-*O*-acetylated sialoglycoproteins and gangliosides using influenza C virus. Anal. Biochem. 194, 425–432.

Manuguerra, J.C., Hannoun, C. and Aymard, M. (1991b) Demonstration of the occurrence of influenza C virus infection in France. J. Infect. 24, 91–99.

Miettinen, T. and Takki-Luukkainen, I.T. (1959) Use of butyl acetate in determination of sialic acid. Acta Chem. Scand. 13, 856–857.

Nishimura, H., Sugawara, K., Kitame, F., Nakamura, K. and Sasaki, H. (1987) Prevalence of the antibody to influenza C virus in a northern Luzon highland village, Philippines. Microbiol. Immunol. 31, 1137–1143.

Nishimura, H., Sugawara, K., Kitame, F. and Nakamura, K. (1988) Attachment of influenza C virus to human erythrocytes. J. Gen. Virol. 69, 2545–2553.

O'Callaghan, R.J. and Labat D.D. (1983) Evidence of a soluble substrate for the receptor-destroying enzyme of influenza C virus. Infect. Immunol. 39, 305–310.

O'Callaghan, R.J., Gohd, R.S. and Labat, D.D. (1980) Human antibody to influenza C virus: its age-related distribution and distinction from receptor analogs. Infect. Immunol. 30, 500–505.

Ohwada, K., Kitame, F. and Homma, M. (1986) Experimental infections of dogs with type C influenza virus. Microbiol. Immunol. 30, 451–460.

Ravindranaths, M.H., Paulson, J.C. and Irie, R.F. (1988) Human melanoma antigen *O*-acetylated ganglioside G_{D3} is recognized by *Cancer antennarius* lectin. J. Biol. Chem. 263, 2079–2086.

Reuter, G., Klotz, F.W., Howard, R.J., Miller, L.H. and Schauer, R. (1991) Influence of sialic acid *O*-acetylation of mouse erythrocyte glycoconjugates on malaria infection. Glycoconjugate J. 8, 224–225.

Rogers, G.N., Herrler, G., Paulson, J.C. and Klenk, H.-D. (1986) Influenza C virus uses 9-*O*-acetyl-*N*-neuraminic acid as a high affinity receptor determinant for attachment to cells. J. Biol. Chem. 261, 5947–5951.

Schauer, R. (1982) The nature of sialic acids. In: R. Schauer (Ed.), Sialic Acids. pp. 32–33. Springer, Wien.

Schauer, R. and Reuter, G. (1988) Metabolism of *O*-acetylated sialic acids. Abstr. Proc. German. Symp. Sialic Acids, pp. 164–165, Berlin.

Schauer, R., Reuter, G., Posadas del Río, F., Herrler, G. and Klenk, H.-D. (1988a) Isolation and characterization of sialate 9(4)-*O*-acetylesterase from influenza C virus. Biol. Chem. Hoppe-Seyler. 369, 1121–1130.

Schauer, R., Reuter, G. and Stoll, S. (1988b) Sialate *O*-acetylesterases: key enzymes in sialic acid metabolism. Biochimie 70, 1511–1519.

Svennerholm L. (1957) Quantitative estimation of sialic acids. Biochim. Biophys. Acta 24, 604–611.

Vlasak, R., Muster, T., Lauro, A., Powers, J.C. and Palese, P. (1989) Influenza C virus esterase: analysis of catalytic site, inhibition, and possible function. J. Virol. 63, 2056–2062.

Warner, T.G. and O'Brien, J.S. (1979) Synthesis of 2′-(4-methylumbelliferyl)-α-D-*N*-acetylneuraminic acid and detection of skin fibroblast neuraminidase in normal humans and sialidosis. Biochemistry 18, 2783–2787.

Warren, L. (1959) The thiobarbituric acid assay of sialic acids. J. Biol. Chem. 234, 1971–1975.

Zimmer, G., Reuter, G. and Schauer, R. (1991) A new method for detection of 9-*O*-acetyl-*N*-acetylneuraminic acid on immobilized glycoconjugates using influenza C virus. Glycoconjugate J. 8, 257.