The Influenza Viruses and Influenza

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Preface

To the biased beholder and student of influenza, it is always an appropriate time to consider its chameleon complexities and review the vagaries of its protean virus. With the present decennial periodicity of pandemics of the disease, it is always just after, between, or just before major changes in the virus.

But now does seem to be a particularly propitious time for a collation of the increasingly abundant data from many laboratories on the biochemistry, structure, and function of the influenza viruses. Not because all the questions have been answered but because the problems have been isolated and better defined—and that is prelude to progress. And although the control of no infectious disease has yet depended upon a definitive understanding of the molecular biology of its causative agent, influenza may well be the first exception. Influenza vaccines, imperfect as they are, are the first to deliberately exploit genetic manipulation in their production. The epidemiology and immunity of the disease are already better understood through the recent clear definition of two discrete viral glycoproteins through which the virion interacts with the host and its environment. Studies of influenza viral structure are well advanced, and understanding of replication is progressing rapidly. On this understanding depends the development of rational approaches to chemoprophylaxis and therapy.

In a real sense, this book began in Madison, Wisconsin in September, 1971, with the first of a series of workshops on influenza sponsored by the National Institute of Allergy and Infectious Diseases. At this first informal conference (with international representation) most of the authors of this book were present, and many also attended the subsequent six conferences during the next three years. At these workshops we got to know each other well and to understand and respect each other's work. The fortunate result has been a continuing active international communication and even collaboration, as is the case with this effort to present a comprehensive view of the influenza viruses and the results of their existence.

Influenza transcends national boundaries so that this present international collaboration seems particularly appropriate.

Edwin D. Kilbourne

Contents

1

The Influenza Viruses and Influenza—An Introduction

E. D. Kilbourne

I. Introduction—Influenza, an Unvarying Disease Caused by a Varying Virus

The extraordinary attention that influenza and its causative viruses have received during the period of modern virology deserves comment in view of the very ordinary symptomatology of this generally uncomplicated infection of the human respiratory tract. The influenza virus replicates in straightforward fashion as a productive infection, without apparent recourse to latency, neoplastic induction, or other subtleties of virus–host relationship, to produce an acute inflammatory disease focused in the trachea that is attended by severe but briefly sustained fever and prostration. The disease in the typical patient has been essentially unvarying during at least the past 400 years, while, paradoxically, it has smoldered as a nuisance, or recurrently has aroused terror as the last great plague of man (Kilbourne, 1963). Paradoxically, also, the disease continues to defy control by artificial immunization despite the proved immunogenicity of specific vaccine

almost 40 years age (Chenoweth *et al.*, 1936). In 1947, with the appearance of the H1N1 strains (Table 1.2), it was first appreciated that the magnitude of antigenic variation of influenza A virus might be so great as to circumvent previously acquired immunity to antecedent strains. Thus, the unvarying disease appears to be caused by a varying virus—indeed, by a virus unique among infectious agents in its antigenic variability. Coincident with the growing evidence of the epidemiologic complexity of influenza, its virus has proved increasingly interesting as an object of laboratory study—the first virus for which hemagglutination was demonstrated (Hirst, 1941; McClelland and Hare, 1941), the animal virus first shown to undergo "recombination" (Burnet and Lind, 1949), the virus first demonstrated to contain an enzyme as a structural protein (Hirst, 1942; Rafelson *et al.*, 1963; Kilbourne and Laver, 1966; Drzeniek *et al.*, 1966), and the virus first shown to reproduce by budding from the plasma membrane of the host cell (Murphy and Bang, 1952). Yet remarkably, research on the virus rarely has been isolated or segregated from research on the disease. The seminal early investigations of the "first generation" of scientists interested in influenza, Shope, Andrewes, Smith, Laidlaw, Stuart-Harris, Hoyle, Mulder, Burnet, Hirst, Francis, Horsfall, and Smorodintsev, have been conducted alternately at the bench and in the field, gaining lessons first from one source and then the other. This tradition has continued to the present so that many of the authors of this book could have written effectively about other aspects of influenza viruses or influenza than those assigned for their special consideration.

The last 10 years have witnessed an accelerated advance in understanding of the structure, replication, and immunology of the virus that has been in part dependent on the application of techniques widely used in molecular biology and in part upon the development of special systems and techniques for study of influenza virus. The latter include the development of plaquing and cloning systems that have facilitated genetic and antigenic studies of the virus (Simpson and Hirst, 1961; Sugiura and Kilbourne, 1965), techniques for isolation and purification of the viral polypeptides (Laver, 1963; Eckert, 1966; Compans *et al.*, 1970; Haslam *et al.*, 1970; Schulze, 1970; Skehel and Schild, 1971), the development of animal models for investigation of pathogenesis and immunity (Shope, 1931; Loosli, 1949; Schulman and Kilbourne, 1963; Nayak *et al.*, 1965; Webster *et al.*, 1971; Potter *et al.*, 1973), and the application of new serologic techniques to viral antigenic analysis and field investigations (see Schild and Dowdle, Chapter 11). The result of this activity has been a considerable increase in knowledge of the nature of the virus, a marked improvement in epidemiologic surveillance, the design of novel approaches to the fabrication of new vaccines (Chapter 15), and, as yet, no substantial impact on the incursions of the disease.

II. Taxonomy of the Influenza Viruses

The influenza viruses are relatively large enveloped viruses that contain RNA in divided or segmented form as genome. The single-stranded RNA molecules are of nonmessage polarity ["negative" in Baltimore's terminology (Baltimore, 1971)] and accordingly contain an RNA-dependent RNA transcriptase within the virion. Transcripts of the five to seven RNA segments code (probably as monocistronic messengers) for the production of five to seven virion structural proteins and possibly cell associated nonstructural protein(s) as well (see Chapters 2, 6, and 8).

As orthomyxoviruses, the influenza viruses bear certain striking similarities to the larger parainfluenza viruses of the paramyxovirus genus (Table 1.1), including the possession of negative stranded RNA and a virion transcriptase, an external envelope that contains glycoprotein spikes bearing

Table 1.1 Taxonomic Relationship of Influenza Viruses (Orthomyxoviruses) to Other RNA Animal Viruses[a]

Family	Genus	"Species"[c]
Picornaviridae	*Enterovirus*	
	Rhinovirus	
	Calicivirus	
Togaviridae	*Alphavirus*	
	Flavivirus	
—[b]	*Orthomyxovirus*	Influenza (type) A
		Influenza (type) B
		Influenza (type) C
—[b]	*Paramyxovirus*	
—[b]	*Coronavirus*	
—[b]	*Arenavirus*	
—[b]	*Bunyamwera* "supergroup" (genus provisional)	
—[b]	*Leukovirus* ("oncornavirus"; "RNA tumor viruses")	
—[b]	*Rhabdovirus*	
Reoviridae	*Reovirus*, *Orbivirus*	"Diplornavirus" suggested

[a] Based on 1971 report of International Committee on Nomenclature of Viruses (ICNV), Wildy (1971) and Fenner *et al.* (1975).

[b] No family designation recognized.

[c] Species designation seems appropriate on basis of absent complementation and recombination among influenza A, B, and C viruses.

Table 1.2 Taxonomy of Influenza Viruses

Family	Genus	"Species" (type)	Subtype[a] (human)	Prevalence	Antigenic variant[b] (within subtype)
None defined	*Orthomyxovirus*	Influenza A	H0N1	(?1929–1947)	Several (not listed)
			H1N1	(1946–1957)	Several (not listed)
			H2N2	(1957–1968)	Several (not listed)
			H3N2	(1968–)	Hong Kong/1/68[d]
					England/42/72
					Port Chalmers/1/73
					Scotland/840/74
			(animal)		
			Hsw1N1[c]		
			Heq1Neq1, etc.		*e*
		Influenza B	No true subtypes but antigenic variation may be equivalent to that observed within an influenza A subtype (e.g., H3N2)		
		Influenza C	None		Significant variation has not been defined

[a] Major antigenic variant with respect to hemagglutinin and/or neuraminidase external virion antigens. Chronologic transition from one subtype to another constitutes antigenic "shift" and is usually associated with pandemic disease.

[b] Minor antigenic variant within a subtype, probably resulting from series of point mutations and selection. May effectively challenge subtype-specific immunity within an interpandemic period. Antigenic "drift."

[c] Probable human prototype strain of 1918–1929.

[d] Strain designation is A/Hong Kong/1/68 (H3N2) [*Bull. W.H.O.* **45,** 119 (1971)].

[e] See Tables 14.1 and 14.2.

hemagglutinating and neuraminidase activity, and respiratory tract pathogenicity. Unlike the parainfluenza viruses, however, the influenza viruses possess a segmented genome, undergo high frequency genetic recombination or reassortment (Chapter 7), have a nuclear actinomycin D-susceptible replication phase, demonstrate no cell fusing or hemolytic properties, appear to contain hemagglutinin and neuraminidase activities in separate proteins, and have not been convincingly shown to cause persistent infections experimentally or in nature.

Influenza viruses exist as three biologically similar, but antigenically heterologous types, A, B, and C (Table 1.2). These viruses share no virus-coded antigens in common, differ in epidemiology and probably to some degree in the severity of illness that they cause. Genetic recombination, or complementation, among these types has not been verified. Therefore, the types appear to deserve identification as separate viral species (see footnote *c* to Table 1.1), *Subtypes* of influenza A virus comprise the major antigenic variants that when they first appear are associated with pandemics. Only one such subtype is present in the human population at any one time—each being superseded by the next in turn. In the case of animal influenza viruses, antigenically different subtypes can coexist (Chapters 9 and 15).

In man, significant variation in both neuraminidase and hemagglutinin antigens of a subtype is recognized every two to three years during interpandemic periods. Such variants probably exist as a continuum based on sequential point mutations of the original prototype strain (Chapter 10). However, rapid strain selection in nature plus inadequacies of sampling creates a picture of rather limited variation which, however, is sufficiently different among strains for their categorical definition. In each period of subtype prevalence such recognizable minor variants have appeared. Table 1.2 lists only the four minor variants of the contemporary H3N2 (Hong Kong) subtype that have thus far been associated with significant widespread epidemic disease. Such variants possess some antigenic determinants in common, but differ completely with respect to others (Laver *et al.*, 1974). The revised taxonomy of influenza virus strain designations appears in Table 1.3.

III. A Comparison of Influenza A, B, and C Viruses—Similarities and Differences

Soon after the isolation of influenza A virus from human disease, it became evident that not all cases of influenza were associated with that virus. In 1940, Francis (1940) and Magill (1940) independently isolated a virus

Table 1.3 Antigenic Characteristics of Reference Influenza A Viruses[a,b]

Neuraminidase subtypes	Hemagglutinin subtypes													
	H0	H1	H2	H3	Hsw1	Heq1	Heq2	Hav1	Hav2	Hav3	Hav4	Hav5	Hav6	Hav7	Hav8
N1	1	2			5										
N2			3	4									14		
Neq1						6		8	10						
Neq2							7							15	
Nav1										11	12				
Nav2												13			
Nav3								9							
Nav4															16
Nav5													17		

[a] Revised from tabulated data from *Bull. W.H.O.* **45,** 119 (1971).

[b] Previous designations:

1 = AO/PR/8/34
2 = A1/FM/1/47
3 = A2/Singapore/1/57
4 = A2/Hong Kong/1/68
5 = A/swine/Wisconsin/15/30
6 = A/equine-1/Prague/56
7 = A/equine-2/Miami/63
8 = A/FPV/Dutch/27
9 = A/turkey/England/63
10 = A/chicken/Germany N/49
11 = A/duck/England/56
12 = A/duck/Czech./56
13 = A/tern/South Africa/61
14 = A/turkey/Mass./65
15 = A/duck/Ukraine/1/63
16 = A/turkey/Ontario/6118/68
17 = A/Shearwater/E. Australia/1/72

from human influenza that differed completely antigenically from influenza A virus. This virus, which resembled influenza A viruses in host range and biological behavior, was termed influenza B virus. Still later, in 1947, Taylor (1949) recovered a hemagglutinating virus from a subject suffering from mild upper respiratory tract infection. "Strain 1233" was originally thought to be a sporadic isolation of a virus of little public health importance, but Francis and his associates (1950) later isolated an antigenically identical virus from an epidemic of respiratory disease in children. Subsequently, the association of this virus with other episodes of mild influenza-like illness appeared to justify designation of the virus as influenza C. Difficulties in developing adequate tissue culture systems for replication of the virus and the infrequency of its demonstrable association with human disease have curtailed its study, so that much less information is available concerning its properties than is the case with influenza A and B viruses. At this writing, the presence of a viral neuraminidase has not been verified (Kendal and Kiley, 1974), nor has any other glycosidase been related to destruction of its cellular receptors (Table 1.4) that follows hemagglutination reactions. It seems probable that neuraminidase or other glycosidase activity will be found when appropriate experimental conditions have been defined.

The order of discovery of influenza A, B, and C viruses is also the rank order of these viruses with respect to their human and animal virulence, their host range in cell culture, the magnitude and frequency of antigenic variation, their optimal replication temperature, and their epidemiologic importance (see Table 1.4). Influenza A viruses differ importantly from B and C in their existence and serial transfer in lower animal species and in their association with pandemic disease—two factors that may be associated (Chapters 10 and 15). An interesting unexplained susceptibility to amantadine also differentiates influenza A from influenza B and C viruses, although some amantadine derivatives are reported to affect influenza B virus (Indulen *et al.*, 1974). An actinomycin D-sensitive step early in influenza A viral replication (indicative of a need for cellular DNA transcription) has not yet been unequivocally established for influenza B or C viruses.

Perhaps reflecting its lesser virulence and apparent predilection for the upper respiratory tract, influenza C virus differs from influenza A and B viruses in replicating (at least in the chick embryo or *in vitro* infection) most efficiently at less than mean human body temperature (37°C). Possibly this virus, and to some extent influenza B virus, are "shut off" from replication at the higher temperature of the human lung in a manner similar to selected temperature-sensitive mutants of influenza A virus (Mills and Chanock, 1971) and consequently are reduced in primary human pathogenicity.

Table 1.4 Comparison of Influenza A, B, and C and Their Causative Viruses[a]

	A	B	C
Virus first isolated	1900[b]	1940	1947
Viral structure	5–7 negative strand RNA segments 5–7 structural proteins including RNA polymerase-enveloped virus	Probably same as A, but less studied	Possession of viral neuraminidase is uncertain
Antigenic variation	Major and minor	Minor	Very minor
Replication and inhibition	Actinomycin D-sensitive step, inhibited by amantadine	? (but mitomycin C inhibition)[c] Not inhibited by amantadine	? ?
Optimal replication temperature	37°C	35°C	32°C
Genome and genetics	Segmented; high frequency recombination	Probably segmented; high frequency recombination	Nature of genome and frequency of recombination not known
Host range (experimental)	Chick embryo, ferret, hamster, subhuman primates, mice, abortive (neurovirulent) replication in mouse brain; chick embryo, avian, primate, and bovine cell cultures	Same as A except for lack of demonstrated experimental neurovirulence	Chick embryo, ferret, hamster, monkey; chick embryo, and primate cell cultures
Host range in nature	Man, various other vertebrates[d]	Man (rarely and sporadically others)	Man
Disease in man	Principally middle respiratory tract, possibly more virulent than B and C in adult	Principally middle respiratory tract, possibly more severe than A or C in child, as in Reyes syndrome	Principally upper respiratory tract, less severe than A or B
Epidemiology	Pandemic, epidemic, and endemic forms in all age groups Present in man at least since 1889[e] May exist and persist in sub-human mammals and birds	Epidemic and endemic phases; disease most common in older children Present in man at least since 1935[e]	Subclinical or endemic infection must be common Present in man at least since 1936[e]

[a] Note that some of the apparent differences among influenza A, B, and C viruses may simply reflect less adequate study of B and C.
[b] First recovery of fowl plague virus from chickens (Stubbs, 1965). First isolation of a probable human virus was from swine in 1931 (Shope) and from man in 1933 (Smith, Andrewes, and Laidlaw).
[c] Nayak and Rasmussen (1966).
[d] See Chapter 14.
[e] Based on serologic studies.