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Influenza: Viral Determinants of the Pathogenicity and Epidemicity of an Invariant Disease of Variable Occurrence

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Influenza: viral determinants of the pathogenicity and epidemicity of an invariant disease of variable occurrence

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If influenza is a riddle wrapped in mystery inside an enigma, then the viral genes are the riddle, the variable surface antigens for which they code are the mystery, and the course and cause of epidemics the ultimate enigma. Paradoxically, the disease itself has remained a stable and recognizable entity through the years, whether initiated by A/PR/8/34 or A/USSR/90/77 variant viruses. Thus, evolution appears to have preserved the disease but not the virus. Among the questions before us are: (1) Have we become obsessed with differences instead of similarities, and have we overemphasized minor differences in viral (antigenic) structure as epidemic determinants? (2) To what extent do viral antigens reflect selection by population antibody? (3) To what extent is antigenic change the pleiotropic consequence of protein structural alteration for purposes other than escape from specific neutralization? These and other questions are discussed in relating viral form to function.

A decade has passed since H3N2 (alias Hong Kong) influenza virus appeared in 1968 to fulfil the tenuous promise of 1957 that major antigenic variants of influenza A virus would emerge 10 years or so coincident with the disappearance of their predecessors. And now, although in 1979 the prophecy appears to be near realization with rather indolent replacement of H3N2 with virus of H1N1 subtype, the prophets among us are chastened by what we had failed to predict, the premature and abortive appearance of swine influenza virus in transmissible form and the premature recycling of H1N1 virus. Thus, although the times may not be out of joint, the viruses *are*, in the light of current doctrine.

We must re-examine our hypotheses in the light of recent epidemiological events and the rapidly increasing knowledge of the influenza viruses attested to by this meeting.

Influenza: invariant disease

Descriptions of ancient influenza are clearly recognizable as descriptions of the modern disease as well. 'Invariance' here applies to the usual or typical case in which cell necrotizing infection is confined to the respiratory tract leading to the abrupt onset of an acute pharyngotracheitis attended by fever, prostration and myalgia disproportionate to objective clinical signs or manifest pathological change. As with all infections, a distribution curve of disease severity is observed in every epidemic ranging from asymptomatic to fatal infections. The larger the epidemic (or the more astute, or perhaps uncritical, the investigator) the more atypical and aberrant manifestations of infection are remarked, including encephalitis, myositis and even parotitis. In relating disease variability to variation in intrinsic viral virulence, one is confounded not only by the variation in human genotype, the importance of which is increasingly recognized by the immunologist, but peculiarly, with influenza, the changing substrate of

immunophenotypes which the virus must infect in order to survive. When, as in the past, the genotype of the infecting virus has been unknown, then observations of the interaction of poorly defined virus and inadequately characterized host have been remarkable in demonstrating the constancy of the clinical expression of infection resulting from such interaction.

While acknowledging the complexity and limitations of our present techniques of genetic analysis of the virus, characterization of the recycled H1N1 viral genome by oligonucleotide mapping by Palese and his colleagues may offer an unprecedented opportunity to observe the impact of prospectively genotyped virus on populations different in time and immune state (table 1). Yet even in this natural experiment, which provides a unique opportunity to evaluate the effects of naturally induced heterotypic and homotypic immunity, the virus has, as usual, been uncooperative in presenting us in 1977 with a virus demonstrably different in nucleotide sequence from the initially invading virus of 1947. Thus, the recycling seems to be inexact in having chosen for re-entry into the population, this time, virus more similar to 1950 than 1947 strains (Nakajima *et al.* 1978).

TABLE 1. EFFECTS OF H1N1 VIRUS ON POPULATIONS OF DIFFERENT IMMUNE STATES

1946		1977	
H1N1 virus		H1N1 virus	
↓		↓	
population		population	
	Hsw1N1 antibody	no heterotypic or homotypic antibody	Hsw1N1 antibody
H0N1 antibody	H0N1 antibody		H0N1 antibody
			H1N1 antibody
< 20 years	> 20 years	< 25 years	> 25 years
↓	↓	↓	↓
influenza	*influenza*	*influenza*	*no disease*†

† In epidemic numbers.

However, we are not yet prepared to distinguish between the 1947, 1950, or 1977 viruses by significant differences in their clinical expression in man. They all cause influenza. I am not persuaded that H1N1 viruses cause a milder influenza on a case-by-case comparison than we have seen with the other viral subtypes. If, for the sake of discussion, I can pursue the difficulty of assessing natural viral virulence even under the present reasonably well defined conditions, let me point out that even those under 20 years old in 1947 had had heterotypic priming with the antigenically close H0N1 viruses (Meiklejohn & Bruyn 1949), and those who were older had encountered Hsw1N1 as well (Davenport *et al.* 1953). Therefore, if disease in the immunologically inexperienced young of 1977 is not demonstrably more severe than in 1949, perhaps we can surmise that the new virus may be less intrinsically virulent than that of 1947. It is also remarkable that the 1947 virus spread as well as it did in a partly immune population. But, on the other hand, H0N1 vaccines failed to provide significant heterotypic immunity. High attack

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rates occurred in 1947–8 in American military populations (Sartwell & Long 1948), an increase in pneumonia was observed in infants (Stuart-Harris 1949), and H1N1 virus was isolated from fatal pneumonia of adults (Maxwell *et al.* 1949). A coincidental finding not relevant to my main thesis is the solidity of homotypic immunity after 20–30 years which has already influenced the epidemiology of 1977–9.

I have gone through this tortuous exercise to illustrate the great difficulties of deriving valid inferences concerning viral virulence in Nature even with the present utilization of molecular virology. If the H1N1 virus causes disease which in fact is milder than other influenza, then clear evidence for this point has not been published (Kilbourne & Loge 1950). Indeed, a recent report cites two fatal cases in older children in the winter of 1978/9 (Schapp *et al.* 1979).

TABLE 2. INFLUENZA A VIRUS HUMAN PATHOGENICITY GRADIENT

			transmissible in Nature			epidemic	
virus	infective for man	can disease man	to man	man to man	sequentially	regionally	globally
H0N1	+	+	+	+	+	+	+
H1N1	+	+	+	+	+	+	+
H2N2	+	+	+	+	+	+	+
H3N2	+	+	+	+	+	+	+
H3N2 (A/HK/5/72)†	+	+	+	+	+	+	0
Hsw1N1 (Fort Dix)	+	+	+	+	+	0	0
Hsw1N1 ‡ (other)	+	+	+	0	0	0	0
Hav1N1 (?)	+	+	+	0	0	0	0
Heq2Neq2	+	+	0	0	0	0	0
other avian viruses	0	0	0	0	0	0	0

† Variants of other human subtypes could be cited.
‡ Also attenuated live virus vaccines.

INFLUENZA A VIRUSES AND HUMAN PATHOGENICITY

If virulence reflects the properties of the virus that enable it to replicate and damage cells, then pathogenicity encompasses, in addition, the ability of virus to spread from man to man in successive generations to produce local or generalized epidemics (table 2). Indeed, strains representative of all of the human subtype viruses can do this very effectively. But, granting the evidence that all mammalian influenza viruses appear capable of infecting and diseasing man, there seems to be a gradient of pathogenicity that can be distinguished.

Thus, variants such as the H3N2 strain A/HK/5/72 caused regional epidemics but were not generally epidemic (Schild *et al.* 1973), and the Fort Dix swine influenza virus was clearly capable of sequential transmission in man (Top & Russell 1977) but was epidemically abortive. At a still lower level of pathogenicity, other swine influenza viruses appear capable only of single step crossing of the species barrier from swine to man. An equivocal case of fowl plague infection of man, not associated with laboratories, has been reported, and Heq2Neq2 influenza virus has produced infection and disease in man when artificially administered in large dosage. With the single exception mentioned, the avian influenza viruses, for all that they may share some gene products with human viruses, have not yet demonstrably infected man.

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I conclude from all this that: (1) mutants probably exist in most influenza A viruses that are capable of productive replication in man, and (2) with animal viruses the ensuing production of virus is either quantitatively or qualitatively insufficient to ensure the effective transmission of virus upon which epidemics depend. Virulence and transmissibility are, of course, viral properties clearly separable in experimental infections with mice (Schulman & Kilbourne 1963). Productive infection implies that cell-to-cell transmission occurs within the host. Therefore, only enhancement of total virus yield by any gene or gene combination may be necessary for epidemicity.

Influence of a single mutation on viral pathogenicity

I should like to turn now from these generalities, which I offer only as reminders of the complexities of pathogenicity, to a specific example of the influence on pathogenicity of a single mutation in a single viral gene.

Table 3. Linkage of three phenotypes to L–H haemagglutinin mutation (Kilbourne 1978)

virus	RNA gel 'genotype' 1 2 3 4 5 6 7 8	viral yield	inhibition by A/sw/Cam/39 antiserum	plaque size in MDCK cells/nm
A/NJ/11/76(L)	SSSS SSSS¶	16‡	20§	1–2.5 (clear)
X-53	PPPS PSPP‖	512	20	1–3
X-53-PR8	PPPS PPPP	1024	20	1–3
A/NJ/11/76(H)	SSSS †SSSS	128	< 10	2–4 (turbid)
X-53a	PPPS†PSPP	4096	< 10	2–5
X-53a-PR8	PPPS†PPPP	8192	< 10	2–5

1–8: ordered sequence of viral RNAs by decreasing molecular mass. RNAs 1–3 code for P (polymerase) proteins, 4 for haemagglutinin, 5 for NP, 6 for neuraminidase, 7 for M and 8 for NS (non-structural) protein.

† Mutation of haemagglutinin gene not detected by RNA gel migration but only by phenotype.
‡ Haemagglutinin titre.
§ Reciprocal of serum dilution at endpoint.
¶ S: migration of RNA on polyacrylamide gel characteristic of A/NJ/11/76 virus.
‖ P: migration of RNA on polyacrylamide gel characteristic of A/PR/8/34 virus.

In 1976 isolates of swine influenza virus from either swine or man, two populations of antigenically distinct particles were demonstrable. Their initial identification in my laboratory depended on the extent of low level reactivity with antiserum to the A/sw/Cam/39 strain of swine influenza virus. When haemagglutinin and neuraminidase genes were segregated from other swine influenza virus genes by recombination with PR8 virus, the antigenic differences persisted as well as other pleiotropic characteristics that distinguished the mutants: (1) yield in chick embryos and (2) plaque size in MDCK cells (see table 3). Further segregation of the swine haemagglutinin gene alone through back-recombination with PR8 demonstrated that pleiotropic phenotype persisted and therefore was attributable to mutation in the haemagglutinin (HA) gene (Kilbourne 1978).

Clearly, this mutation influenced viral replication in chick embryo and MDCK cells. Does it have any pathogenic significance for swine, the natural host? In preliminary experiments, B. Easterday and I have found that indeed different replication patterns are associated with the L (low yielding) and H (high yielding) HA phenotype, whether or not the gene is borne by

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cloned wild-type virus or segregated in the PR8 recombinants (table 4). In either low or high dosage, nine out of ten pigs were infected with the L wild-type virus and excretion of viruses was protracted. Equivalent egg infective doses of the H variant were less infective and even after high dosage of virus were less frequently recovered. Similar patterns were observed with the L and H PR8 recombinants demonstrating (1) the apparent determination of more efficient replication in swine by the L haemagglutinin; (2) less efficient replication associated with the H genotype which, paradoxically, determines *high* yield in the chick embryo and MDCK cells; (3) the conversion of PR8 from a virus apparently unable to replicate in swine to one that can by the substitution of a single gene; and (4) the additive effect of non-haemagglutinin swine influenza virus genes on viral replication in swine (table 5).

TABLE 4. DETERMINATION BY A SINGLE HAEMAGGLUTININ MUTATION OF INFECTIVITY OF WILD-TYPE AND RECOMBINANT VIRUSES FOR SWINE

inoculum		no. of pigs	virus
virus	dose†	infected	excretion‡
A/NJ/11/76(L) (SSSSSSSS)	10^2	5/5	18/40
	10^5	4/5	19/32
A/NJ/11/76(H) (SSSS*SSSS)	10^2	1/5	1/40
	10^5	2/5	7/40
X-53-PR8(L) (PPPSPPPP)	$10^{5.7}$	5/5	14/40
X-53a-PR8(H) (PPPS*PPPP)	$10^{5.6}$	0/5	0/40
A/PR/8/34 (PPPPPPPP)	$10^{6.3}$	0/5	0/40

S is the A/NJ/11/76 (Hsw1N1) influenza virus gene; the 4th gene codes for haemagglutinin; S* is the 'H' mutant. P is the A/PR/8/34(H0N1) influenza virus gene. (L) and (H) are low and high yielding mutants, respectively of A/NJ/11/76(Hsw1N1) influenza virus (Kilbourne 1978). Data from Kilbourne *et al.* (1979).

† E.i.d.$_{50}$.

‡ Number of pigs per group multiplied by the number of days on which virus was isolated.

Because the L and H variants are virtually indistinguishable in reciprocal HI tests it seems unlikely that one is an immunoadaptive mutant of the other. Rather I conclude that mutation of the haemagglutinin has been selected for on the basis of changed replicative capacity, and that the associated minor antigenic change is essentially fortuitous. This may be so with currently emerging H1N1 mutants such as the Brazil prototype that apparently arose in South America in populations of non-immune young people, but which is now in the ascendancy in the United States (*Morbidity and Mortality Weekly Report* 1979). Other variants equally different antigenically will probably not survive (like A/HK/5/72), either from bad fortune or because their antigenically manifest change in haemagglutinin structure has failed to enhance concomitantly replication, virulence or transmissibility.

The early return of H1N1 to fill the void created by the gradual subsidence of H3N2 viruses may indicate the limits of structurally tolerable variation of the haemagglutinin in viruses

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capable of producing epidemics. Certainly other haemagglutinins in nature are antigenically better qualified to challenge immunity of the population than is H1.

Of course, more than the haemagglutinin gene will determine the epidemic potential of the virus. Table 6 summarizes these requirements for an epidemic virus and provides tentative gene assignment to steps in replication and viral transmission. I hope that it is useful in summarizing our present ignorance of the molecular determinants of influenza, the disease. Probably I should rather say it summarizes *my* ignorance. If the picture is more clear than as represented here I hope that corrections and additions can be made during discussion.

TABLE 5. VIRAL REPLICATION IN NATURAL HOST (SWINE) AND LABORATORY HOST SYSTEMS IN RELATION TO HAEMAGGLUTININ PHENOTYPE

viral genotype†	chick embryo	MDCK cells	swine
SSSSSSSS(L)	+	+	+ + +
PPPSPPPP(L)	+ +	+ +	+ +
SSSS†SSSS(H)	+ +	+ +	+
PPPS†PPPP(H)	+ + +	+ + +	0
PPPPPPPP(PR8)	+ + +	+ + +	0

† See footnote to table 3.

TABLE 6. REQUISITES OF INFLUENZA VIRUS VIRULENCE AND THEIR PROVISIONAL DETERMINATION BY VIRAL GENES

ability of viral haemagglutinin to bind to target cells (HA)
ability of virus to infect target cells (HA cleavage by host cell proteases; may be influenced by NA)
ability to replicate productively (P1, P2, P3, NP, NS) + host
ability to replicate at a *rate* sufficient to achieve adequate proportion of *stable* infective virus to achieve intercellular spread (M and probably others)
ability to effect release of virions to facilitate intercellular spread (NA)
ability to attach to infect and damage non-respiratory tract cells to cause extra-pulmonary 'complications'
[ability to cause sufficient cellular damage to ensure expulsion (and transmission) of virus (any or all genes)]†

† Not properly a virulence attribute except to the extent that evolution and maintenance of virulence requires repeated host transfer and multiple viral generations.

DISCUSSION AND SUMMARY

Despite the mutability of influenza viruses and the variable substrate of human immunophenotypes resulting from response to its successive invasions, the interaction of virus and host throughout the years has resulted in disease of remarkable constancy with respect to mean severity. The host and its defences are the adapting filter through which the virus must force the appropriate mutant, able to infect respiratory tract epithelium, to replicate to high titre with production of sufficient stable, infective virus to damage cells and ensure its serial expulsion and transmission to other hosts. Although a wide variety of viral genotypes appear capable of causing epidemic influenza, I expect that further genetic analysis will define the boundaries of allowable variation and that highly conserved nucleotide sequences common to all such strains will be found. Furthermore, we shall have to look more critically at the potential of animal virus antigens to function in human infections than merely to ascertain their degree of antigenic

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difference from human viruses. With further knowledge, the list of candidate 'pandemic' antigens may diminish.

On the other hand, minimal variation, as with a single point mutation, may be critical in determining whether or not a virus will infect, cause disease, or produce epidemics. Present knowledge of influenza virus biology (molecular or otherwise) provides us with no satisfactory explanation for the epidemiologic events of 1976 and 1977, nor does it yet tell us why people get sick when infected with the virus.

As we dissect out the viral genetic components of virulence in laboratory systems, we must be wise enough to be cautious in extrapolations to human disease and bold enough continually to attempt correlations as knowledge increases.

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