

Recent Contributions of Molecular Biology to the Clinical Virology of Myxoviruses

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Recent advances in the clinical virology of influenza are based on non-pragmatically oriented research on the genetics and biochemistry of the influenza virus. Antigenically hybrid recombinant viruses can be tailored to provide monospecific reagents for serological studies. Basic research on viral structure and the mechanism of viral replication has directly influenced the establishment of a cell culture system suitable for the isolation of most influenza viruses. Identification of viral genotype by RNA gel electrophoresis and mapping of oligonucleotides of viral RNA has already facilitated epidemiologic investigations. The clinical virologist of the future must have an understanding of the potential limitations of these techniques for specific strain identification.

Rather than review the well established and well known procedures employed in the isolation and identification of influenza viruses, I should like to take this opportunity to describe some recent developments in basic research on these viruses that have already had practical application to clinical diagnosis. In addition, I shall suggest some implications for the future of clinical virology of current research on the molecular genetics of influenza viruses.

THE USE OF PROTEASES IN INFLUENZA VIRUS-HOST CELL SYSTEMS— A NEW STRATAGEM FOR VIRAL ISOLATION

The isolation of influenza viruses until recently has required the use of embryonated eggs or primary or secondary monkey kidney cell culture—systems not readily available in many laboratories. Other primary cell systems have been cumbersome and have not received wide acceptance. The aneuploid cell lines so convenient for the isolation of other human viruses do not support the replication of influenza viruses in general [1]. The first aneuploid cell found to be permissive for influenza virus replication, the Wong-Kilbourne mutant of the Chang conjunctival cell line [2,3] was relatively insusceptible to many influenza viruses. A neglected paper by Gauth and Smith in 1968 [4] described plaque formation by a number of influenza A and B viruses in a canine kidney cell line (MDCK) established by Madin and Darby [5]. Despite this discovery of a relatively permissive cell system, MDCK cells proved variable in sensitivity to many viral strains.

The final solution to the problem came from a combination of empirical observation and basic studies of viral structure and function. The incorporation of trypsin in overlay media had been found to enhance influenza virus plaque formation by an

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undefined mechanism [6-9]. Unrelated studies of viral structure demonstrated that endogenous cleavage of the viral hemagglutinin by endogenous proteases in the infected host cell was required for optimal infectivity of the virus particle [10,11]. Furthermore, addition of exogenous trypsin to virus with uncleaved hemagglutinin resulted in increased infectivity of such preparations [12]. It is now clear that the enhancing effect of trypsin on plaque formation is the result of its action on the hemagglutinin of the budding virus—not on the cell per se. Thus, when MDCK cells, the most permissive of cells available, are overlaid with trypsin-containing media, a highly permissive and sensitive host cell system is available for the cultivation and primary isolation of influenza viruses [13]. Recent studies have shown that the MDCK cell-trypsin system may be more sensitive than either eggs or Rhesus monkey cells for isolation of currently circulating influenza A and B strains. Sufficient hemagglutinin was produced on the initial tissue culture passage to allow direct identification of isolates by hemagglutination-inhibition tests. The same study demonstrated that a variety of other respiratory viruses also replicated in MDCK cells so that over a ten-month period 35 percent of 600 specimens yielded virus in this system [14].

I have detailed the development of this virus isolation system only to make what I think is an important and perhaps neglected point. That is, the importance of modifying environmental and cultural conditions as well as cell type before concluding that presently available cells are unsusceptible to viruses. Although intrinsic cellular susceptibility is indeed important (even with the MDCK cell-trypsin system) the implication of the trypsin story is that other appropriate manipulations of cultural conditions (changes in pH, incubation temperature, etc.) should be explored, in addition to searching for new cell types. Furthermore, the annoying variability in susceptibility encountered with cells already used in viral diagnostic work can be addressed by cloning of these ordinarily heterogeneous cell populations [2].

THE USE OF RECOMBINANT VIRUSES IN CLINICAL VIROLOGY

Influenza viruses possessing segmented RNA genomes readily undergo recombination during coinfection of cells in the laboratory with different strains of the same type [15]. By the use of appropriate selective systems, new viruses with the required characteristics that are uniquely suited for serologic studies can be isolated. Thus, antigenic hybrids can be engineered to possess the hemagglutinin (HA) antigen of one parental virus and the neuraminidase (NA) of the other [16,17]. Such recombinants are effectively monospecific antigenically when used in measurement of antibody response to virus identical to one or the other parent. For example, the level of anti-hemagglutinin antibody to the H1 antigen following infection with H1N1 influenza virus (the currently circulating subtype) is most accurately determined using a recombinant virus that does not bear an N1 neuraminidase (NA) antigen (e.g., H1N2) and hence is not sterically inhibited by the combined effects of anti-hemagglutinin and anti-neuraminidase antibody. Antibody rises so demonstrated may be lesser in magnitude but will be more specific. The great change in the HA antigen (from H2 to H3) in 1968 was not immediately appreciated because the new Hong Kong virus (H3N2) possessed the same (N2) NA antigen as the antecedent Asian (H2N2) strain. Antibodies to H2N2 virus were cross-reactive with H3N2 through the common NA antigen and obscured the magnitude of antigenic differences between the H2 and H3 hemagglutinin components. When antisera monospecific for the hemagglutinin were prepared with antigenic hybrids in which H2 hemagglutinin was segregated from the N2 NA [18] it became clear that the new Hong Kong virus was essentially a

composite of an "old" NA and a "new" HA and therefore a possible natural recombinant [19]. Epidemiologic considerations aside, diagnosis of the individual case and identification of viruses during the past decade of HK prevalence has been facilitated by the use of HA-specific and NA-specific reagents—now routinely provided by the CDC and WHO. NA-specific antigenic hybrids have also been employed in vaccines to induce NA-specific immunity [20,21,22].

POTENTIAL CONTRIBUTION TO THE MOLECULAR EPIDEMIOLOGY OF INFLUENZA VIRUSES BY THE BIOCHEMICAL CHARACTERIZATION OF INFLUENZA VIRUS GENES

In 1973, I used the term "molecular epidemiology" [23] to review the contribution of more precise antigenic and biochemical characterization of influenza viruses to an understanding of their epidemiology. As mentioned earlier, the recognition of the Hong Kong (H3N2) pandemic virus as more than just another "A2" strain came from such analysis.

Since that time, a technique had been developed that permits the definition of distinctive patterns of extracted viral RNAs on polyacrylamide gel electropherograms and makes possible comparisons among viruses with respect to all 8 genes of the virus [24,25]. This method demonstrated that the virus isolated from the 1976 epidemic of influenza at Fort Dix was indistinguishable from contemporary swine influenza virus strains and therefore had most likely originated from swine [26]. Although this method will continue to be useful in comparisons and categorization of new isolates, it usually is inadequate to pick up single or point mutations that may be critical in determining the virulence or transmissibility of a virus, unless such base changes affect secondary RNA structure and therefore migration patterns on gels. A case in point is two swine influenza virus hemagglutinin mutants which have identical RNA gel patterns but marked differences in biological properties, probably determined by a single mutation [27].

The further refinement of oligonucleotide mapping of T-1 ribonuclease digests of influenza viral RNAs offers promise for much more precise strain identification [28,29]. This technique already has revealed a startling similarity of the new Russian H1N1 virus to a 1950 strain with respect to all genes—not just those coding for the surface glycoproteins.

Although hardly applicable to the diagnostic laboratory at present, in the manner of salmonella typing, these refinements of viral technology offer promise of definitive strain identification, perhaps by WHO, state, or medical center laboratories in the future. In related studies, the fingerprinting of herpes simplex virus strains by using restriction endonucleases originally devised for mapping of the viral DNA has already contributed to the tracing of transmission of HSV in a new-born nursery [30] and may solve legal questions concerning the source of genital infections [31].

No longer is the clinical virologist content to identify an isolate merely as influenza A, B, or C. In the future he will contribute to the identification of regionally or locally prevalent strains of virus and will participate therefore even more than in the past in defining influenza viral epidemiology and disease patterns.

NOTE ADDED IN PROOF

Oligonucleotide mapping of recent 1978–79 influenza A viruses has shown that one such virus prevalent in the United States is a natural recombinant of H1N1 and H3N2 viruses that co-circulated in man the previous year [32].

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