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MONKEYPOX VIRUS AS A POTENTIAL SOURCE OF VARIOLA VIRUS

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Summary

Genome mapping by restriction endonucleases is a powerful method of examining the relationships between orthopoxviruses, and gives useful results at the levels of species, strains and mutants. Genome analysis amply confirms the biological evidence that monkeypox, variola, vaccinia, cowpox and ectromelia are distinct species. Some mutants of monkeypox obtained by one of us (SSM) had previously been reported to have the biological characters associated with "whitepox" or variola virus. DNA analysis confirmed that six of these isolates had also a genome structure like that of variola; it is difficult to see how the many necessary changes in the monkeypox genome could have been brought about. An intensive study has been organized and 21 mutants of monkeypox obtained by JHN and KRD have now been analysed. These showed several kinds of alteration in the monkeypox genome, but all of the altered genomes were recognizable as monkeypox. Further clarification is required, but on present evidence we conclude that species barriers are not readily crossed and that the variety of mutants derivable from monkeypox in the laboratory does not present any greater threat to the success of smallpox eradication than does the existence in nature of orthopoxvirus species other than variola.

Introduction

Monkeypox virus was first isolated in 1958 (1), from an outbreak of a generalized poxvirus infection in cynomolgus monkeys that had been shipped from Singapore to the Statens Seruminstitut in Copenhagen. Over the next decade a number of similar incidents occurred, and virus isolations were reported on four occasions (2). The virus took on a new significance when it was recognized in 1970 to be the cause of a human disease clinically resembling smallpox (3,4) which has now been found to occur as a rare sporadic zoonosis among villagers living in tropical rainforest areas of west and central Africa (5).

One of us (SSM) has recently reported that viruses indistinguishable from variola virus by laboratory tests were obtained from certain laboratory stocks of monkeypox virus, either by pock selection from infected chorioallantoic membranes (CAM), or by passage in hamsters (6,7). These observations were held to offer a possible explanation for the origin of "whitepox" viruses, a term which has been used to refer to six viruses which were isolated from animals and which also are indistinguishable from variola virus by laboratory tests (8).

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The derivation of a variola-like virus from monkeypox would seem to be inherently unlikely. In addition to several biological markers which distinguish these two viruses (see Table 1) major biochemical differences have been reported in comparison of polypeptides produced in infected cells (9). Even more important are recent analyses of the DNA of variola and monkeypox viruses by means of restriction endonucleases (10,11). Mackett and Archard (11) have constructed maps of the cleavage sites of three restriction endonucleases on two strains of variola virus and three strains of monkeypox. The differences between the two viruses proved to be both numerous and located in several different regions of the genome. Conversion of monkeypox into variola would require a number of separate alterations in the structure of the monkeypox virus genome.

Prior to 1978 the variants of monkeypox had received only scant attention. Three isolates which produced white pocks on the CAM had been recorded. One retained the parental characteristics of virulence for chick embryo and rabbit skin (12) and this and another white pock isolate had the monkeypox-specific antigen (13). A third was like the parent in chick embryo virulence and ceiling temperature (14). Each was thus readily distinguishable from variola virus by biological tests (see Table 1).

If true, the derivation of a virus indistinguishable from variola virus from monkeypox virus would have serious implications for the Smallpox Eradication Programme of the World Health Organization (8), since it suggests that monkeypox virus, which appears to be widespread among primates and perhaps other wild animals in west and central Africa (5), is a potential source from which human smallpox could re-emerge.

The Biological Properties of Monkeypox, Variola and Related Viruses

Table 1 summarizes a number of biological properties that characterize four kinds of orthopoxvirus: variola virus, monkeypox virus, "whitepox" virus, and several white pock mutants of monkeypox virus recovered independently by several investigators (12,13,14; K.R. Dumbell, unpublished data; J.H. Nakano, unpublished data). In respect of the biological and chemical properties examined, they fall into two groups: on the one hand, variola virus and "whitepox" virus are essentially identical; on the other, monkeypox virus and its white pock mutants show minor differences from each other but are clearly different from variola virus.

Comparison of the DNA Maps of Several Species of Orthopoxvirus

A large central part of the linear DNA molecule of all species of orthopoxvirus appears to be highly conserved (11,15,16); variations between strains and the larger variations between species reside mainly in the nucleotide sequences near the terminal regions of the genome. This type of structure has permitted the construction of a dendrogram (Figure 1) that shows the relatedness of 16 viral strains belonging to five species of orthopoxvirus, obtained by analysing the data provided by Mackett and Archard (11) and rating the relative site positions according to the computer program MULCLAS (17) to derive a numerical index of dissimilarity.

With this programme, the 16 strains form five clusters, which correspond to the five species as defined by their biological characteristics: monkeypox, variola, vaccinia (including rabbitpox), ectromelia and cowpox viruses. The highest dissimilarity rating within a species is 0.75, for monkeypox strain Congo, recovered from a human case in Africa in 1970 compared with monkeypox virus recovered from cynomolgus monkeys in Denmark in 1958. The dissimilarity ratings between species, on the other hand range from 2.06 (monkeypox and variola viruses) through 2.23 (vaccinia and ectromelia viruses) to 2.82 (cowpox virus and the other four species).

FIGURE 1

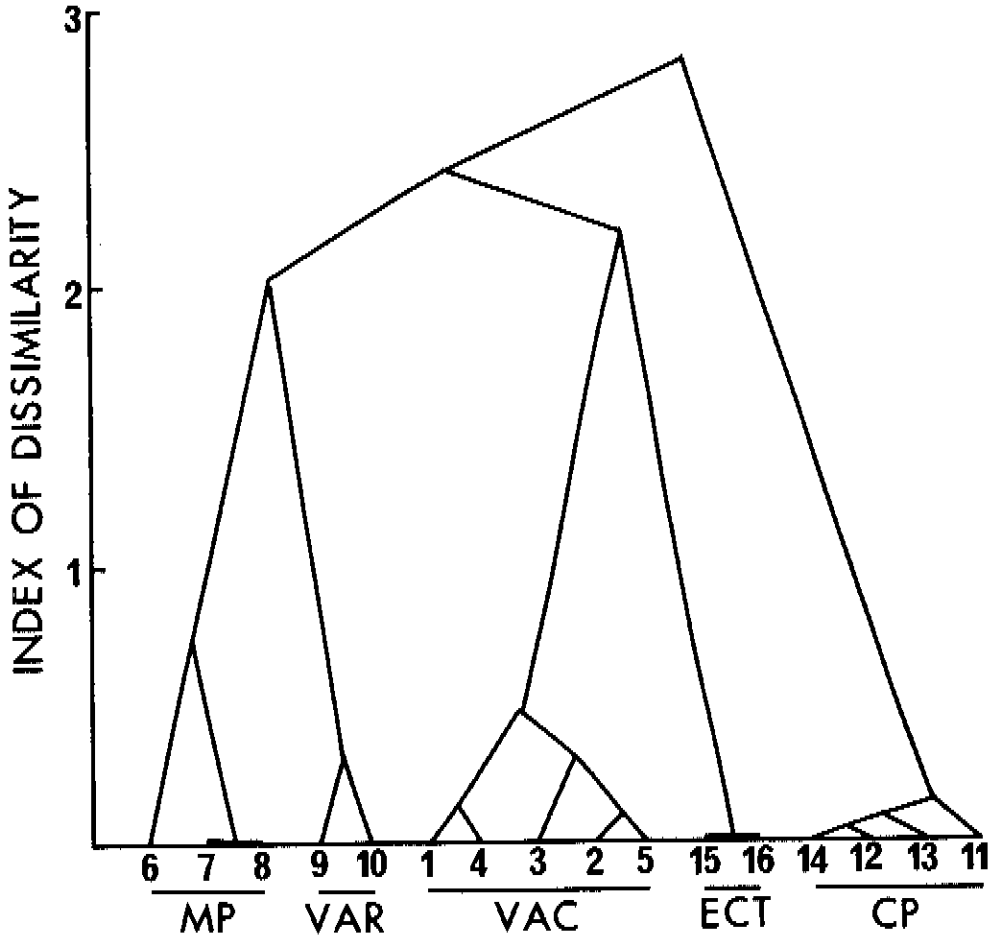

Figure 1 is a dendrogram illustrating the relatedness of 16 strains of orthopoxvirus, obtained by subjecting the similarities and differences in the cleavage sites of three restriction endonucleases (Sma I, Hind III and Xho I), as reported by Mackett and Archard (11), to analysis in the computer program MULCLAS (17). Euclidian metric used to assess similarity of individuals, Burr's sorting strategy to produce a dendrogram.

Key

MP = monkeypox virus, 6 = Congo, 7 = Denmark, 8 = Espana.

VAR = variola virus, 9 = Butler (minor), 10 = Harvey (major).

VAC = vaccinia virus, 1 = rabbitpox Utrecht, 4 = Lister, 3 = Hall Institute, 2 = DIE, 5 = Western Reserve.

ECT = ectromelia virus, 15 = Hampstead, 16 = Moscow.

CP = cowpox virus, 14 = Daisy, 12 = Brighton, 13 = Ruthin, 11 = Austria.

Considering the very different origins and histories of the orthopoxvirus strains (variola minor and major, monkeypox from man and monkey in different continents, vaccinia strains of very different passage histories, cowpox and ectromelia viruses from different countries in Europe) the "within-species" differences are remarkably small compared with the "between-species" differences. These results were obtained by analysing the maps of cleavage site location for the restriction endonucleases Hind III, Sma I and Xho I. Analysis of data from other laboratories, using Hind III, Sac I, Sal I and Bam HI, gave comparable results (15, J.J. Esposito, unpublished results). These findings suggest to us that it is highly unlikely that any one of these species of virus could be changed into another species by one or a few mutational steps. Of course, we would not deny the possibility that certain of these species, as we now find them, may have been more closely related to each other in the historic past.

White Pock Mutants of Orthopoxviruses

All orthopoxviruses that produce haemorrhagic ulcerated pocks on the chorioallantoic membrane have been found to produce a small proportion (usually 0.1% to 0.5%) of white pocks (cowpox (18); rabbitpox (19,20); neurovaccinia (21); monkeypox (12)). DNA analysis of three white pock mutants of the Brighton strain cowpox virus (22) showed that their genomes had a substantial deletion at the right hand end, but white pock mutants of other strains of cowpox showed a wider spectrum of alterations (Dumbell and Archard, unpublished observations). Several white pock mutants of rabbitpox virus have also revealed sequence deletions, some mutants from the right and some from the left hand end of the molecule (23,24).

White Pock Mutants of Monkeypox Virus

Two of our laboratories (Atlanta and London) have been engaged in DNA analysis of orthopoxviruses. Preliminary observations have been made on a number of white pock mutants recovered on the CAM.

Four white pock mutants were isolated and charaterized in Atlanta from two strains of monkeypox virus (J.H. Nakano and J.J. Esposito, unpublished observations). All four had lost all or most of the pathogenicity for rabbit skin shown by the parent and one had developed some ability to grow in a pig kidney cell line (see Table 1). Maps were constructed of the distribution of cleavage sites for two restriction endonucleases (Hind III and Sac I). The genome of one isolate showed a deletion in the right terminal region and concomitant rearrangements of terminal sequences. Maps of two others showed no detectable differences from the parental monkeypox map. The fourth isolate was similar to the first but more complex changes were detected in the genome of this virus. All four isolates could still be identified with parental monkeypox virus.

In London, 11 white pock clones isolated from one strain of monkeypox have been fully characterized (Dumbell and Archard, unpublished observations). Four biological markers were used, including the white pock. Four of the mutants were like monkeypox except for the white pock, five were like monkeypox in only two of the four markers and two were like variola in all but one of the biological markers used. Cleavage site maps were constructed for three restriction endonucleases. Three of the mutants showed major deletions at the right hand end of the genome. The two which were phenotypically most like variola showed rearrangements involving both ends of the molecule but despite these multiple changes still did not show the genome structure of variola virus. Others had similar rearrangements involving both ends of the molecule and two further mutants showed no detectable change from the parental genome. Six other white pock clones have been isolated but not fully characterized. None of them, however, is variola-like in either biological characters or genome structure. The factors which lead to the different kinds of genomic change are still unknown and it is clear that there can be significant changes in phenotype which are not reflected in the cleavage site maps so far available.

DNA analysis has also been undertaken in Atlanta of four white pock isolates recovered from the CAM in Moscow (6), and in London of two mutants recovered from hamsters in Moscow (7). All six of these isolates had genomes whose cleavage site maps were not distinguishable from those of variola (unpublished data of J.J. Esposito and of L.C. Archard) thus confirming the identification made on the basis of biological tests (6,7). These six isolates had been obtained from uncloned parental stocks of two strains of monkeypox. In DNA analysis, one of these stocks was typical of monkeypox but the other gave a complex pattern in the DNA electropherograms, explicable only as a mixture of fragments from both variola and monkeypox viruses (J.J. Esposito unpublished data).

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