Epidemiol. Infect. (1999), **122**, 287–290. Printed in the United Kingdom © 1999 Cambridge University Press

A variant of variola virus, characterized by changes in polypeptide and endonuclease profiles

K. R. DUMBELL,[1,2,3]* L. HARPER,[4] A. BUCHAN,[4] N. J. DOUGLASS[3] AND H. S. BEDSON[4]†

[1] *St Mary's Hospital Medical School, University of London, UK*
[2] *PHLS Centre for Applied Microbiology and Research, Salisbury, UK*
[3] *Department of Medical Microbiology, University of Cape Town, RSA*
[4] *Department of Medical Microbiology, University of Birmingham, UK*

(*Accepted 24 September 1998*)

SUMMARY

A variant of variola virus is described which produces a late polypeptide of 25 kDa instead of one of 27 kDa and which has an additional endonuclease cleavage site for *Sal*I in the viral DNA. These markers were shown to be genetically independent and to characterize 14 of the 48 variola strains which were examined. The variant strains were isolated from smallpox outbreaks originating in or from Pakistan between 1961 and 1974 and also from two cases at a Mission Hospital in Vellore, India in 1964. No variant strains were found among 9 other isolates from cases of variola major occurring in other parts of India or in Bangladesh, nor among 4 isolates from Indonesia, 15 from Africa or 6 isolates of variola minor.

INTRODUCTION

The final destruction of all remaining stocks of variola virus is expected before the end of this century. Before that deadline it is important that all information pertinent to the study and understanding of variola viruses should be placed on permanent record.

Variola viruses have been found to be remarkably constant in their properties, wherever or whenever they had been isolated. The late polypeptides, pulse-labelled 16 h post infection, have a characteristic pattern which distinguishes variola from vaccinia, cowpox and monkeypox viruses [1]. Variola viruses showed a polypeptide of approximately 27 kDa as a characteristic feature of their pattern. Here we show that a minority of variola strains produced a polypeptide pattern that lacked the 27 kDa band but had instead a polypeptide of 25 kDa.

Variola viruses have also been characterized by the distribution of cleavage sites within their genomic DNA for various restriction endonucleases. Esposito and colleagues [2] reported that the DNA of each of two viruses (7155, 7275) that had been isolated from monkey kidney tissue culture in Holland had a *Sal*I fragment which was smaller than the corresponding fragment in three strains of variola virus. Dumbell and Kapsenberg [3] showed that these two strains and two strains of variola originating in 1964 from Vellore, India had an extra endonuclease cleavage site resulting in a truncation of the *Sal*I L fragment, normally 5·5 kbp, by about 0·5 kbp.

Here we report the occurrence of variola major viruses which exhibited both the truncated *Sal*I L DNA fragment and the 25 kDa polypeptide.

MATERIALS AND METHODS

Viruses

Some of the variola viruses were isolated from cases of smallpox by one of us (K. R. D.); others were obtained from the late Drs A. W. Downie, A. Herrlich, J. H.

* Corresponding author: present address: PO Box 1933, Somerset West, 7129, South Africa.
† Henry Bedson died in 1978 but was closely involved in the planning and execution of the polypeptide work.

https://doi.org/10.1017/S0950268899002150 Published online by Cambridge University Press

Nakano and M. S. Pereira, and from Dr S. S. Marennikova.

Virus strains 7255, 7275, CH9 and MK7 were isolated from animal tissues but all were indistinguishable from variola major virus, both in biological properties and in DNA analysis. Two of them (7255, 7275) isolated in Holland have been shown to be laboratory contaminants with variola virus from southern India [3], but all four are regarded as genuine variola viruses [4]. These four strains, collectively dubbed the 'whitepox' viruses, were made available through the World Health Organization.

Methods

The studies involving culture of variola virus were made between 1977 and 1982 at the Medical School, Birmingham University, at the Virology Department, St Mary's Hospital Medical School, London and at the Centre for Applied Microbiology and Research, Salisbury; DNA sequencing studies were done at the Department of Medical Microbiology, University of Cape Town.

Preparations of late viral polypeptides were obtained and used as described by Harper and colleagues [1]. Experiments using this technique were performed at Birmingham in 1977 and 1978 by H. S. B., L. H. and A. B.

Viral DNA was prepared and restriction digests analysed as described by Dumbell and Kapsenberg [3]. A DNA library of the Vellore, 1964 strain 7124 was prepared [3], by K. R. D. during 1981–2, while working at CAMR, Porton Down, Salisbury. DNA sequencing was done at the Medical Microbiology Department of University of Cape Town in 1991–2 by N. J. D. and K. R. D., as previously described [5].

RESULTS

Fourteen strains of variola have been tested for both the 25 kDa polypeptide and the truncated *Sal*I L DNA fragment. The results are shown in Table 1. Five strains showed both the 25 kDa and the truncated *Sal*I L DNA fragment; the other nine were normal in both characters. Even when the two strains 7255 and 7275 are taken as identical the odds against this being a chance correlation would be 714:1. This strong evidence of correlation might, of course be due to the two tests detecting the same genetic alteration. To determine whether or not the two markers were independent, it was decided to sequence relevant parts of Vellore 7124 DNA from a DNA library of this strain which had been constructed at CAMR some years previously [3].

Table 1. *Variola viruses examined for occurrence of a polypeptide of 25 kDa and for a truncated* Sal*I L fragment of viral DNA*

Data isolated	Isolate	Origin of specimen	Peptide (kDa)	*Sal*I L (kbp)
1944	Harvey	Britain	27	5·5
1958	BOM	India	27	5·5
1962	MAD 1	India	27	5·5
1962	MAD 2	India	27	5·5
1967	628	Kuwait	25	5·0
1987	629	Kuwait	25	5·0
1969	NAS	Pakistan	25	5·0
1970	ZAI	Zaire	27	5·5
1972	ETH	Ethiopia	27	5·5
1974	SHZ	Bangladesh	27	5·5
1972	CH9	Moscow*	27	5·5
1975	MK7	Moscow*	27	5·5
1964	7255	Bilthoven*	25	5·0
1964	7275	Bilthoven*	25	5·0

* These are the four isolates of variola viruses from animal tissues, described in the Materials section.

In the variola strain Harvey the 5·5 kDa *Sal*I L fragment maps toward the right-hand end of the *Hin*dIII A fragment [6]. This region of Harvey DNA was sequenced by Aguado and colleagues [7] and in their sequence *Sal*I L runs from nucleotide 8591 to 14089. It is this 5·5 kDa L fragment (incorrectly labelled K in ref. [3]) which is truncated in the variant strains to a size of approximately 5·0 kDa. It is intriguing that, in Harvey, the right-hand end of *Sal*I L lies near the 3′ end of the ORF designated 21R by Aguado and colleagues [7] which encodes a predicted polypeptide of 27·6 kDa.

A recombinant plasmid containing a *Sal*I insert of approximately 5·0 kbp was identified in the Vellore 7124 library and DNA sequence was obtained inwardly from both ends. Sequence from the one end was identical to a part of the Harvey sequence to the right of nucleotide 8591; the other end matched Harvey sequence to the left of nucleotide 13546 [7], confirming that the truncated *Sal*I fragment in the variant corresponds to the left end of Harvey *Sal*I L between nucleotides 8591 and 13546. To confirm this, a cloned plasmid containing a *Sal*I fragment of about 0·5 kb was identified from the 7124 DNA library and some sequence obtained from either end of the insert. Sequence at one end was identical to Harvey DNA 3′

https://doi.org/10.1017/S0950268899002150 Published online by Cambridge University Press

Table 2. *Chronological list and place of origin of isolates of variola virus showing either or both the 25 kDa and the truncated* Sal*I L markers*

Area	Locality	Dates	Variant isolate	25 kDa	*Sal*I L
UK*	Leeds	1961	McC	+	−
	Cardiff	1962	SHU	+	−
India	Vellore	1964	7124	−	+
	Vellore	1964	7125	−	+
Kuwait*	Kuwait City	1967	K5/67	+	−
	Kuwait City	1967	1628,	+	+
	Kuwait City	1967	1629	+	+
Pakistan	Lahore	1969	NAS	+	+
Iran†	Teheran	1971	2602	+	−
	Teheran	1972	9866,	+	−
	Teheran	1972	9879,	+	−
	Teheran	1972	9883	+	−
Pakistan	Rural village	1973	JUM	+	−
	Karachi	1974	KAR74	+	−

* Originated from Karachi, Pakistan.
† Originated from Afghanistan.

to nucleotide 13551 [5], confirming that this fragment represented the truncated portion of the Harvey *Sal*I L. The additional *Sal*I cleavage site will thus lie within the coding sequence of the Harvey 21R ORF [7]. However the new *Sal*I cleavage site results from a single T-C conversion of the Harvey sequence. This change would not alter the amino acid encoded in that triplet and would not result in a truncation of this presumptive polypeptide. Hence it can be concluded that the 14 variant strains of variola shown in Table 1, are each identifiable by two independent markers.

The strains shown to bear both variant markers had been isolated from cases occurring over 5 years (1964–9) and a wide geographic area. Further work confirmed that either marker was found in only a minority of variola viruses. As strains bearing the same two independent markers are highly unlikely to have arisen often, it seemed reasonable to regard the isolates as part of a sustained transmission of a single variant strain of variola. It follows that it would be reasonable to record the date and place of origin of all the variant strains identified by at least one of the two markers. The results of this are shown in Table 2.

One strain that was found to possess the 27 kDa polypeptide has been excluded from the results. This strain (Abid) was obtained from another laboratory at what was said to be the fourth passage on chick chorioallantois (CAM) of a Pakistan specimen. We found that this strain did not produce the expected hyperplastic foci in HeLa cells [8, 9] but gave rise to multiple syncytia. Only two other variola strains, to our knowledge have behaved like this and in each case the strain had been passed more than 35 times in series on the CAM. In addition, this Pakistani strain produced a small number of pocks on the CAM of eggs incubated at 39 °C. Again we have not met any early passage variola isolate which would do this. We therefore doubt the stated provenance of this strain, and have not included any results obtained with it in this record.

DISCUSSION

No variants were found among five isolates from India (Bombay, Calcutta and Madras, 1958, 1962, 1971) or among four isolates from Indonesia (1969 and 1970).

Fifteen isolates made between 1960 and 1977 from various countries in Africa (Ethiopia, Somalia, Tanzania, Sierra Leone, Zaire and Botswana) all lacked a variant marker, as did six strains of alastrim virus isolated in Brazil, UK and The Netherlands.

Four isolates of variola major occurring in Britain (1944, 1946, 1958), one of which was believed to have been introduced from Bombay, lacked a variant marker. However two variola major isolates in Britain (1961, 1962) were of the variant phenotype; the index case in both outbreaks had arrived from Karachi, one in December 1961 and the other in late January, 1962 [10].

https://doi.org/10.1017/S0950268899002150 Published online by Cambridge University Press

The first detected instance of the variant was thus the isolation of two strains from the smallpox imported into Britain in 1961 and 1962. Then the variant was detected in isolates from an outbreak in Kuwait in 1967, where the first case had arrived from Karachi 2 weeks before she became ill [11]. All of four isolates from cases in Iran in 1971 and 1972 were of the variant type. The smallpox occurring in Iran at that time had been introduced by a family who had arrived from Afghanistan in October 1970 [12]. All of three isolates originating directly from Pakistan were of the variant type. They were from Lahore in 1969, from a rural village in 1973 and from Karachi in 1974.

Thus all the variant isolates, except those from Vellore, appear to have originated from Pakistan or Afghanistan between 1961 and 1974. As it is highly improbable that a variant exhibiting the same two independent markers would occur twice it can be presumed that transmission of the variant strain was sustained in Pakistan and Afghanistan at least for the 13 years covered by the variant isolates reported here. No isolate of the normal type was found among the isolates originating from Pakistan, but the numbers are so small that it is not possible to speculate what proportion of the variola occurring in Pakistan between 1961 and 1977 might have been of the variant phenotype. It was noted above how smallpox had reached Iran and Kuwait; there is no information about a traveller going from Pakistan to Vellore, but it is possible that the Christian Mission Hospital in Vellore had received an out of state visitor who was incubating the disease.

ACKNOWLEDGEMENTS

It is a pleasure to acknowledge expert technical assistance from Ruth Reith at St Mary's Hospital Medical School and Basil Maber at the Centre for Applied Microbiology and Research.

The studies done at the University of Birmingham and at St Mary's Hospital Medical School were supported by the World Health Organization. Facilities for the experimental work at CAMR, Porton were made available by the Public Health Laboratory Service of the United Kingdom. The work at the University of Cape Town was supported by a grant from the Poliomyelitis Research Foundation of the Republic of South Africa.

REFERENCES

1. Harper L, Bedson HS, Buchan A. Identification of orthopoxviruses by polyacrylamide gel electrophoresis of intracellular polypeptides. Virology 1979; **93**: 435–44.
2. Esposito JJ, Obijeski JF, Nakano JH. Orthopoxvirus DNA: Strain differentiation by electrophoresis of restriction endonuclease fragmented virion DNA. Virology 1978; **89**: 53–66.
3. Dumbell KR, Kapsenberg J. Laboratory investigation of two 'whitepox' viruses and comparison with two variola strains from southern India. Bull WHO 1982; **60**: 381–7.
4. Fenner F, Wittek R, Dumbell KR. The orthopoxviruses. San Diego: Academic Press, 1989: 221–4.
5. Douglass NJ, Richardson M, Dumbell KR. Evidence for recent genetic variation in monkeypox viruses. J Gen Virol 1994; **75**: 1303–9.
6. Hamilton and colleagues. Unpublished results quoted in Fenner F, Wittek R, Dumbell KR. The orthopoxviruses. San Diego: Academic Press, 1989: 208.
7. Aguado B, Selmes IP, Smith GL. Nucleotide sequence of 21·8 kbp of variola major virus strain Harvey and comparison with vaccinia virus. J Gen Virol 1992; **73**: 2887–902.
8. Vieuchange J, de Brion G, Gruest J. Virus variolique en cultures de cellules. Ann Inst Pasteur. 1958, **95**: 681–93.
9. Pirsch JB, Purlson EH. A tissue culture assay for variola virus based upon the enumeration of hyperplastic foci. J Immunol 1962; **89**: 632–7.
10. U.K. Ministry of Health, Reports on Public Health and Medical Subjects, No. 109. Smallpox 1961–62. London: HMSO, 1963.
11. Arita I, Shafa E, Kader MA. Role of hospital in smallpox outbreak in Kuwait. Amer J Pub Hlth 1970; **60**: 1960–6.
12. Fenner F, Henderson DA, Arita I, Ježek Z, Ladnyi ID. Smallpox and its eradication. Geneva: WHO, 1988: 1089.

https://doi.org/10.1017/S0950268899002150 Published online by Cambridge University Press