563

Molluscum contagiosum: characterization of viral DNA and clinical features

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SUMMARY

Restriction endonuclease analysis of molluscum contagiosum virus DNA revealed two subtypes. In a study of 46 isolates from 41 patients, some with no other disorder and some with atopic dermatitis, the ratio of MCV I isolates to MCV II was 34:12. Multiple clustered lesions removed at the same time from an individual patient yielded only one type of MCV. Lesions induced by MCV I or MCV II were indistinguishable on the basis of size and form. Neither subtype was associated exclusively with lesions at certain sites or with other clinical features. Heterogeneity of DNA restriction endonuclease cleavage patterns amongst isolates of the same subtype was observed, this being greatest for MCV II.

Molluscum contagiosum, a benign skin tumour of man resulting from a common poxvirus infection, has not previously been investigated intensively because of the inability to culture the virus *in vitro* and the fact that ordinarily the papules resolve spontaneously. Transmission of the virus is mechanical, and lesions may be found anywhere on the skin but are usually clustered. A common site of infection in adults is the genital area and, in such cases, sexual transmission of the virus is likely. Numerous, widespread, persistent and disfiguring lesions may develop in the immunosuppressed (for example in Hodgkin's disease or AIDS) and in atopic dermatitis, where immune defects have been suggested as a possible explanation for the increased incidence of molluscum contagiosum and other cutaneous infections (Postlethwaite, 1970; Brown, Nalley & Kraus, 1981). Restriction endonuclease analysis of MCV DNA has revealed two viral subtypes (Darai *et al.* 1986). We have reported the further characterization and physical mapping of their genomes, which are essentially collinear, and shown that MCV DNA is similar to Orthopoxvirus DNA with respect to size, terminal cross-linking and the presence of inverted terminal repetitions (Porter & Archard, 1987).

In the study described here we have characterized viral DNA from 46 independent isolates from 41 individuals presenting to the skin or genito-urinary clinics to see whether there is any correlation between MCV type and clinical features or the distribution of the lesions. When several lesions were clustered at the same site and might reasonably be assumed to have arisen from the same infection, lesions were pooled. Patients with or at risk of human immunodeficiency
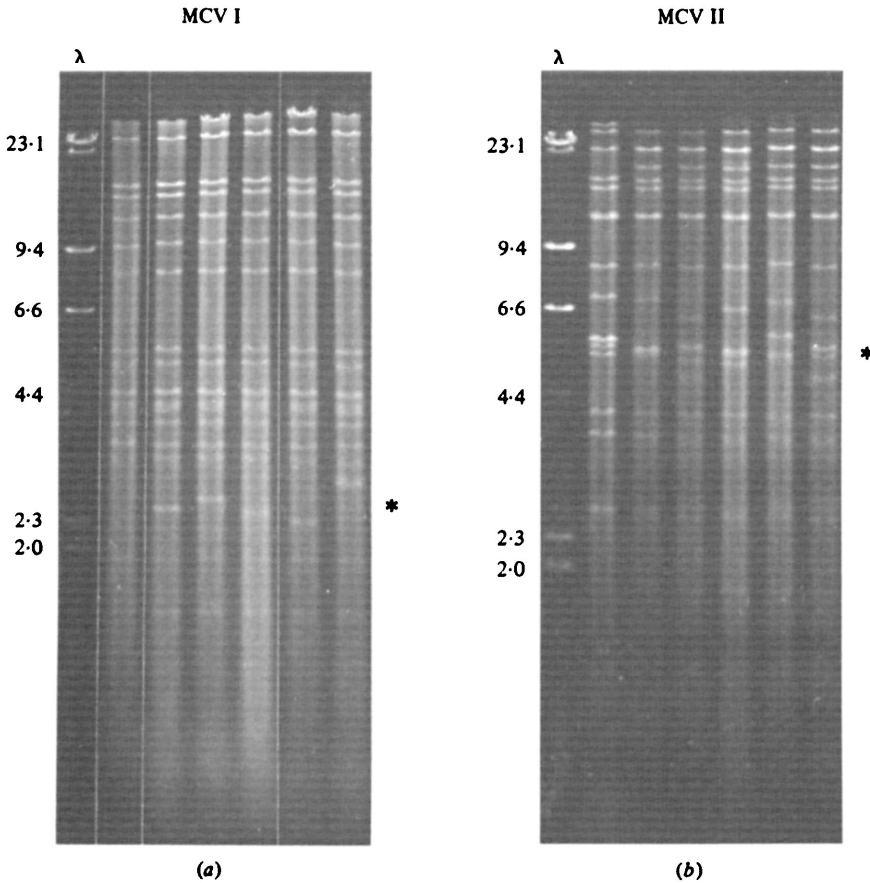
Fig. 1. Restriction endonuclease HindIII digests of DNA from six independent isolates of (a) MCV I or of (b) MCV II. Size markers are HindIII fragments of λ DNA: sizes are indicated in kilobase pairs. * indicates the region of the gel in which the terminal fragments (which are 2-molar as HindIII cleaves within the inverted terminal repeat) migrate: size variation between isolates is clearly seen.

virus infection were excluded from the study. Lesions were either curetted or their virus-rich cores were expressed. They were chopped, lysed and digested in 100 mM Tris-HCl pH 7·5, 10 mM EDTA, 70 mM-2-mercaptoethanol, 0·1% SDS, 1% sodium *n*-lauryl sarcosinate, 0·5 mg/ml proteinase K for 16 h at 55 °C. In selected cases lesions were homogenized and virus was purified as described (Porter & Archard, 1987); the virus pellet was then lysed and digested as above. Total DNA from lesions, or purified viral DNA, was deproteinized by extraction with phenol and recovered by ethanol precipitation according to standard methods (Maniatis, Fritsch & Sambrook, 1982). DNA was dissolved in 10 mM Tris-HCl pH 8·0, 1 mM EDTA and cleaved with restriction endonucleases BamHI, ClaI or HindIII. The resulting fragments were separated by agarose slab gel electrophoresis and visualized by ethidium bromide staining and ultraviolet light transillumination.

Restriction endonuclease analysis of DNA from individual isolates revealed two major distinguishable cleavage patterns (Fig. 1). The fragments of viral DNA are easily discerned above a background staining due to the presence of host-cell

Table 1. *Summary data on patients from whom MCV I and MCV II was recovered*

	Patients (isolates)	Sex		Age range (years)	Genital lesions	Atopic dermatitis
MCV I	29 (34)	M	12 (14)	3–31	2 (2)	3
		F	17 (20)	3–62	8 (9)	1
MCV II	12 (12)	M	1 (1)	73	1 (1)	0
		F	11 (11)	16–53	5 (5)	1

Figures in parentheses are numbers of isolates rather than numbers of patients.

DNA. On the basis of these distinguishable cleavage patterns we refer to the two subtypes as MCV I and MCV II. We have previously established a close relationship between them by demonstrating that their genomes cross-hybridize extensively (Porter & Archard, 1987). Some variations in restriction endonuclease cleavage patterns of MCV DNA were observed amongst different isolates of the same subtype, although isolates were always recognizable as MCV I or MCV II (Fig. 1). In particular the size of terminal fragments was variable, as is commonly observed for poxviruses (Wittek *et al.* 1978). Also, occasional loss of individual restriction sites was deduced from the absence in DNA cleavage patterns of two DNA fragments that map adjacently, and the presence of a new fragment corresponding to the sum of their sizes. Such a loss of a restriction site would result from the mutation of a single base pair within the hexanucleotide recognition sequence. The BamHI restriction site located at 75 kilobases from the left terminus of MCV I was missing from a number of isolates (data not shown). Terminal fragment size variations and restriction site loss were the only variations seen in the genomes of 34 MCV I isolates. In contrast, MCV II isolates showed greater variability in DNA cleavage patterns, although summation of restriction fragment sizes indicated that the genome sizes of these isolates were similar (Fig. 1); all were clearly distinguishable from MCV I and DNA cleavage patterns.

Data relating to patient sex, age, lesion site and other clinical features are summarized according to MCV subtype in Table 1. Patients were aged 3–73 years: 13 were male and 28 were female. Seventeen isolates were from genital lesions, 14 of which were from female patients. This bias in favour of females with genital lesions reflects the preponderance of female patients studied from the genito-urinary clinic. Five patients had atopic dermatitis: additionally, one of these had ichthyosis and one had pancytopaenia.

Overall, 34 isolates were MCV I (14 male, aged 3–31 years and 20 female, aged 3–62 years) and 12 were MCV II (1 male, aged 73 years and 11 female, aged 16–53 years). No incidence of mixed infection was found. Eleven isolates were from 10 patients aged less than 16 years (8 male and 2 female), but none of these was MCV II. Only 1 of 13 male patients had lesions containing MCV II although 11 of 28 female patients did so. Considering only the group of patients presenting to the skin clinics (13 male and 17 female) there were 7 isolates of MCV II, of which 6 were from female patients. Of the 5 isolates from patients with atopic dermatitis, 4 were MCV I and 1 was MCV II.

One patient had lesions removed from separate sites on the same visit and processed independently, and four further patients had lesions removed on two

successive occasions. In four cases both isolates were MCV I. However, in the fifth case the first isolate was MCV I and the second, removed from the same site 7 weeks later, was MCV II: both isolates were from genital lesions. This patient could have been harbouring both viruses simultaneously in separate lesions, or may subsequently have become infected with MCV II.

In most cases lesions containing MCV I or MCV II were phenotypically indistinguishable. However, one patient who was otherwise well had on her back unusually large and numerous papules which contained MCV II.

Isolates were obtained from lesions taken from a variety of sites including limbs, trunk, head and external genitalia. There is no indication that either virus subtype is restricted to or predominantly associated with any particular site, as has been shown to be the case with herpes simplex virus (HSV) and human papilloma virus infections (Chaney *et al.* 1983; Gissmann *et al.* 1983). In particular we addressed the question as to whether genital lesions, which probably result from sexual transmission of the virus, were associated predominantly with one subtype analogous to the association of HSV2 with this site of infection. Eleven genital isolates were MCV I and 6 were MCV II. Of the 31 isolates from female patients, 5 of 14 genital lesions and 6 of 17 non-genital lesions contained MCV II. Our observations of the occurrence of MCV II differ from those of Darai *et al.* (1986), who found this subtype in only 1 of 14 isolates, from a genital lesion of a female patient.

We conclude that there are two subtypes of MCV which themselves show varying degrees of heterogeneity and that there is no correlation between the site of infection or associated clinical features and the subtype involved.

(Note added in proof: We have now studied a total of 69 isolates (52 MCV I, 16 MCV II) from 60 patients; there is no change in the pattern described).

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