Molluscum Contagiosum Virus: Antibody Responses in Persons with Clinical Lesions and Seroepidemiology in a Representative Australian Population

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An ELISA for molluscum contagiosum virus (MCV) was used to determine the antibody status of 35 adults with clinical infections and known human immunodeficiency virus (HIV) serology and of 357 persons (ages, 1 week–69 years) considered representative of the Australian population. MCV antibody was identified in 77% of persons with molluscum lesions: in 17 of 24 HIV-1-negative persons and in 10 of 11 who were HIV-1-positive. No relationship was evident between the serologic responses and the number of lesions or the duration of infection. The population survey revealed an overall seropositivity rate of 23%. The lowest antibody prevalence was in children aged 6 months to 2 years (3%), and seropositivity increased with age to reach 39% in persons ≥50 years old. These findings indicate that MCV infections, including very mild or subclinical cases, may be more common in the general community than previously suspected.

Materials and Methods

Patients and specimens. The 35 adult subjects (ages, 19–56 years) with clinically diagnosed molluscum contagiosum attended sexually transmitted disease (STD) and dermatology clinics in the Sydney region from March to September 1993. The only selection criteria were the availability of lesion material and serum, knowledge of human immunodeficiency virus (HIV) status (established by HIV-1 ELISA and immunoblot serology), and patient consent. Twenty-four were HIV-1-negative (17 men, 7 women) and 11 were HIV-1-positive. No relationship was evident between the serologic responses and the number of lesions or the duration of infection. The population survey revealed an overall seropositivity rate of 23%. The lowest antibody prevalence was in children aged 6 months to 2 years (3%), and seropositivity increased with age to reach 39% in persons ≥50 years old. These findings indicate that MCV infections, including very mild or subclinical cases, may be more common in the general community than previously suspected.

Attempts to elucidate the epidemiology of molluscum contagiosum (MCV) infection by serologic surveys have been restricted by the paucity of available viral antigen, since the agent cannot be propagated in vitro [1] and infections cannot be transmitted to laboratory animals by conventional means [2]. Although various techniques have been used to assess immune responsiveness in lesion-bearing patients [3–5], no investigations into the prevalence of MCV antibodies in the general population have been described. We report the results of an IgG ELISA used to measure MCV antibody responses in 2 groups: the first consisting of patients with clinical molluscum infections and the second considered representative of the general population of Sydney.

The 357 sera investigated in the survey of the Sydney population were from healthy subjects <1 week to 69 years old. These were randomly selected from frozen ‘normal control sera’ originally collected by the New South Wales (NSW) Red Cross Blood Transfusion Service or by various hospital laboratories in the Sydney region as ‘healthy controls’ for other surveys from November 1992 to December 1993. Data on age and sex were available.

MCV 1 ELISA. This assay was performed essentially as previously described [7]. In brief, 96-well ELISA plates batch-coated with purified MCV 1 virion proteins (5 μg/mL) were incubated with blocking solution (1% skim milk powder in PBS for 1 h at 37°C), duplicate aliquots of test or control sera (1:40 in wash buffer) for 18 h at 4°C, peroxidase-conjugated goat anti-human IgG (1:4000 in blocking solution) for 1 h at 37°C, and peroxidase substrate solution for 30 min. Absorbances were read at 690 nm and expressed as optical density units (ODU: mean of the test wells minus the mean of the blank wells). A panel of control sera—2 negative samples and 4 positive samples and 4 blanks (all reagents apart from sera)—were tested on each plate.

Statistical analysis. Data analyses were done by χ² test with Yates’s correction or by a nonparametric test, Spearman’s rank correlation test, where appropriate.

Results

The results of MCV typing on lesions collected from the 35 persons with clinical infection indicated that 23 (66%) contained MCV 1 or 1v, whereas 12 (34%) were positive for MCV 2. The ratios of MCV 1/1v:MCV 2 in lesions from the HIV-negative and HIV-positive groups were 17:8 and 6:4, respectively.

On the basis of our previous investigations [7], a ODU of 0.187 was used as the minimum cutoff value for MCV 1 posi-
Figure 1. Distribution of serologic responses to MCV 1 antigens in 178 adults, expressed as % of sera producing ELISA optical density unit (ODU) readings within specified ranges. Sera with ELISA readings $>0.187$ ODU (vertical line) were classified as MCV antibody–positive. A. Serologic responses of 143 adults 20–49 years old in general population. B. Serologic responses of 35 persons with clinical molluscum infections, including 11 who were human immunodeficiency virus (HIV) antibody–positive.

Table 1. Prevalence of MCV seropositivity in members of the general population of different age groups, showing the ratios of males to females in each grouping.

<table>
<thead>
<tr>
<th>Age</th>
<th>No. (M:F)</th>
<th>MCV antibody–positive (M:F) [%]</th>
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<tbody>
<tr>
<td>0–6 months</td>
<td>13 (8:5)</td>
<td>4 (3:1) [31]</td>
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<tr>
<td>6–24 months</td>
<td>37 (17:20)</td>
<td>1 (1:0) [3]</td>
</tr>
<tr>
<td>2–4 years</td>
<td>46 (24:22)</td>
<td>5 (2:3) [11]</td>
</tr>
<tr>
<td>5–9 years</td>
<td>25 (14:11)</td>
<td>5 (2:3) [20]</td>
</tr>
<tr>
<td>10–19 years</td>
<td>49 (22:27)</td>
<td>9 (4:5) [18]</td>
</tr>
<tr>
<td>20–34 years</td>
<td>84 (45:39)</td>
<td>25 (14:11) [30]</td>
</tr>
<tr>
<td>35–49 years</td>
<td>59 (37:22)</td>
<td>15 (12:3) [25]</td>
</tr>
<tr>
<td>$&gt;50$ years</td>
<td>44 (32:12)</td>
<td>17 (11:6) [39]</td>
</tr>
<tr>
<td>Total</td>
<td>357 (199:158)</td>
<td>81 (49:32) [23]</td>
</tr>
</tbody>
</table>

Overall, by this criterion, 108 (28%) of the 392 sera tested were MCV antibody–positive, including 27 (77%) of 35 from persons with clinical infections and 81 (23%) of 357 sera from the general population. A comparison of the ELISA results with MCV DNA typing indicated that there was no significant difference ($\chi^2$, $P > .5$) between the antibody positivity rates of those who were currently infected with MCV 1/1v (19 [83%] of 23) and those with MCV 2 infections (8 [66%] of 12).

Positive antibody responses were found in 71% (17/24) of the HIV-negative persons with MCV infection, 91% (10/11) of those with MCV infection and coexistent HIV infection, and 28% (40/143) of the adults of comparable age (20–49 years) from the general population. Although there was no difference between the 2 MCV-infected groups ($P > .2$), both had a highly significant ($P < .001$) increased prevalence of antibody positivity compared with the controls. In addition, as illustrated in figure 1, the relative antigenic reactivities (as indicated by ELISA ODU readings) in persons with clinical lesions, regardless of HIV status, were notably higher than in the general population. Almost 44% of the former had ELISA readings $>1.088$, compared with only 7% of control sera.

Reliable data on duration and number of lesions were available for 33 of the 35 persons with clinical infections. Those who were MCV antibody–positive had been clinically infected for periods of 2 weeks to almost 1 year. Of the 6 persons who were negative for both HIV and MCV antibody, 4 had lesions that were present 1–6 weeks before serum collection; in the other 2 subjects, the lesions had been present for 14 and 22 weeks. The mean duration and number of lesions was higher in the HIV-positive group (17.3 weeks, 16.6 lesions) than in the HIV-negative group (13.7 weeks, 8.0 lesions), but these differences were not significant. Analyses of data using Spearman’s rank correlation test also failed to reveal any significant relationships between antibody responses and either duration of infection or number of lesions.

The results of the serologic survey of the general population were stratified according to age groups, as shown in table 1. These divisions were made on the basis of available knowledge regarding the age-related incidence of clinical MCV infection: most common in school-aged children, adolescents, and young adults; less common in preschool-aged children and older adults; rare in young infants. The lowest prevalence of antibody (3%) was in children 6 months to 2 years old. Seropositivity increased with age to a high of 39% in those $>50$ years old ($\chi^2$.001).
trend, $P < .001$). In infants <6 months old, the prevalence was 31%, significantly higher ($P < .02$) than in the slightly older children (6 months–2 years). There was no significant difference ($P > .5$) between the number of males and females who were antibody-positive in any age group (table 1).

**Discussion**

All persons in this study were infected with either MCV 1, 1v, or 2; infections with MCV 3 were not identified, confirming the rarity of infections with this type [6]. One-third of the subjects were currently infected with MCV 2, a figure consistent with our previous Australian survey, which also included a large proportion of patients from STD clinics [6]. Although the antigen used in the ELISA was exclusively MCV type 1 (due to the paucity of type 2 lesional material), we previously demonstrated that there is substantial cross-reactivity between MCV 1, MCV 1v, and MCV 2 in this assay [7], probably due to the presence of shared immunogenic epitopes. However, although our previous investigations showed that all sera positive in the MCV 2–specific ELISA were also positive when tested in the MCV 1 assay, the number of sera in this category was small (18 samples), and the relative sensitivity of the MCV 1 assay for different types has yet to be conclusively determined. It is therefore possible that some of the negative results in MCV 2 lesion–bearing patients may have been due to the inability of the ELISA to detect low levels of MCV 2–specific antibody. It was recently reported that two recombinant MCV polypeptides (70 and 34 kDa encoded by genes MC133L and MC084) are reactive with patients’ sera [8]. The ability to produce type-specific recombinant proteins would overcome the difficulties we experienced in producing purified ELISA antigens in quantity, particularly for the less common types.

We were able to demonstrate MCV antibody in 71% of patients with clinical molluscum infection who were otherwise healthy. This figure is similar to previous results obtained by immunofluorescence [4], although it is considerably lower than the 100% positivity reported by a group using complement fixation on serum samples collected a minimum of 9 weeks after the appearance of lesions [5]. The very lengthy period that can elapse before the appearance of this antibody (>4 months in several subjects in this survey) is related to the sequestering of viral antigens in the upper epidermis and to the fact that MCV can exert a profound local immunosuppressive effect, even in immunocompetent persons [9]. In addition, the observation that many persons had persistent clinical infection in the presence of apparently high levels of MCV antibody supports the concept that elimination of molluscum infection is dependent on the development of an effective cell-mediated immune response [10]. Alternatively, the ELISA may have measured non-protective antibodies directed at irrelevant viral epitopes.

The number of HIV-infected persons in this study was too low to permit a categorization by disease stage, but the clinical data indicated that most (8/11) were significantly immunocompromised (<100 CD4 cells/mm$^3$). It was noteworthy that the prevalence of MCV antibody was highest (91%) in this group. HIV-infected persons are often unable to respond to new antigens; however, they commonly have elevated levels of immunoglobulin (IgG and IgA) because of nonspecific polyclonal activation of B cells [11]. In addition, as molluscum contagiosum is a recognized STD, it is possible that this group had repeated exposure to the virus. The high prevalence and level of antibodies found in these persons could therefore represent either secondary antibody responses or nonspecific rises in immunoglobulins.

It was not unexpected that the general population survey revealed that MCV seropositivity was strongly age-related, since this is similar to the pattern seen in many other viral infections. The high rate (31%) in children <6 years old was considered to represent maternally acquired antibody, since it was very similar to that of adults of childbearing age and strikingly different from the rate (3%) in slightly older children (ages 6 months–2 years). Moreover, clinical MCV infection has been rarely reported in young infants. However, the fact that we found no differences in seropositivity between males and females was somewhat surprising, since it has been reported that the ratio of males to females presenting with molluscum infections is about 2.5:1 [12].

It is difficult for realistic figures on the overall incidence of MCV infection in the general population to be compiled, since it is not a notifiable disease in any country. In temperate regions, its rate has traditionally been considered low, accounting for <1% of all skin infections [13]. However, a recent survey from the Netherlands found the childhood form of the disease to be quite common, with a cumulative incidence of 17% by age 15 years [14]. The adult sexually transmitted form may be less prevalent. Data from New Zealand STD clinics for 1995 indicated that 0.7% of the 30,301 attendees had molluscum contagiosum infection, compared with 8.2% with anogenital warts, 3.7% with herpes simplex virus, 0.5% with chlamydia, and 0.03% with gonorrhea [15]. In this first reported survey of a sample of the general population, the overall MCV seropositivity (23%) was considerably higher than would be expected from estimates based on the numbers of persons seeking medical attention for clinically apparent infections. This discrepancy suggests that subclinical or minor infections must be more common than previously suspected, with resultant antibodies persisting for a long time, if not indefinitely, after infection.

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References