Antibodies to Molluscum Contagiosum Virus in the General Population and Susceptible Patients

Takahiro Watanabe, MD, PhD; Koichiro Nakamura, MD, PhD; Motoshi Wakugawa, MD; Atsushi Kato, PhD; Yoshiyuki Nagai, PhD; Tatsuo Shioda, PhD; Aikichi Iwamoto, MD, PhD; Kunihiko Tamaki, MD

Background: Since many attempts to cultivate molluscum contagiosum virus (MCV) in vitro have been unsuccessful, it is difficult to prepare a large quantity of antigens. To assess the seroprevalence of antibodies against MCV in 508 subjects with or without clinical MCV infection, a truncated recombinant protein from open-reading frame MC133L was synthesized using Sendai virus expression system and applied to enzyme-linked immunosorbent assay as an antigen.

Observations: Antibodies to MCV were present in 7 (58%) of 12 patients with molluscum contagiosum, 7 (6%) of 108 healthy controls, 7 (9%) of 76 with atopic dermatitis, and 7 (18%) of 39 patients with systemic lupus erythematosus, although no clinical MCV infection was observed in the latter 3 groups. Of 7 human immunodeficiency virus (HIV)–positive patients with molluscum contagiosum, 1 (14%) was antibody positive, compared with 5 (2%) of 266 HIV-positive patients without molluscum contagiosum. Serum samples from patients with atopic dermatitis and systemic lupus erythematosus showed a higher reactivity (P<.001) than those from healthy controls, while serum samples from HIV-positive subjects showed a lower reactivity (P<.001).

Conclusion: The humoral immune response to MCV is usually confined to patients with molluscum contagiosum and may be affected by the immunological condition of the host.

Arch Dermatol. 2000;136:1518-1522

MOLLUSCUM contagiosum (MC) consists of waxy, skin-colored, dome-shaped papules measuring 2 to 5 mm and is caused by molluscum contagiosum virus (MCV). It occurs in young children, sexually active adults, and immunocompromised patients. As extensive MC lesions have been found in 5% to 18% of patients with the acquired immunodeficiency syndrome, greater importance has been attached to the virus as a cause of disfigurement. The skin lesions induced by MCV usually persist for months to years, which might be because the virus appears to provoke little cell-mediated immunity. In earlier studies, antibodies to MCV were detected in MC patients using crude antigens from pooled MC lesions or purified virions; the incidence of viral antibodies has been reported as 9 (43%) of 21 cases in the Ouchterlony method, 46 (69%) of 67 in immunofluorescence, and 17 (100%) of 17 in complement fixation. However, an application of these assays to a large-scale survey is hampered since many attempts to cultivate MCV in vitro have been unsuccessful, and it is difficult to prepare a large quantity of antigens. There is clearly a need for enzyme-linked immunosorbent assay (ELISA) using synthetic recombinant proteins as antigens.

Seven major polypeptides have been identified from purified MCV particles by Coomassie brilliant blue staining, and 2 of them, 70- and 34-kd polypeptides encoded by open-reading frame (ORF) MC133L and MC084L, respectively, have been reported to be immunoreactive to sera from patients with MC. In the present study, we selected Sendai virus (SeV) protein recombination system, the expression level of which has been reported to be the highest in mammalian cells, and synthesized a truncated soluble protein devoid of the unique transmembrane anchoring domain of 70-kd polypeptide. We then used this protein as an ELISA antigen and examined the presence of antibodies to MCV in 508 subjects with or without clinical MCV infection.
PATIENTS, MATERIALS, AND METHODS

CELLS AND VIRUSES

The monkey kidney cell lines LLCMK2 and CV1 were grown as monolayers in minimum essential media supplemented with 10% bovine serum. Molluscum lesions were obtained from 44 patients who consulted the Department of Dermatology in Tokyo University Hospital, Tokyo, Japan. Purification of MCV and DNA extraction were performed as previously described. The purity of the preparation was assessed by electron microscopy.

PATIENT SERUM SAMPLES

Serum samples were collected from 12 patients with MC (7 male and 5 female, aged 2.3-9.4 years [mean age, 5.5 years]), 76 patients with atopic dermatitis (AD) (41 male and 35 female, aged 12-37 years [mean age, 18.8 years]), and 39 patients with systemic lupus erythematosus (SLE) (6 male and 33 female, aged 19-62 years [mean age, 31.7 years]) whose disease course had been followed by members of the Department of Dermatology in Tokyo University Hospital between 1992 and 1997. All patients with MC were otherwise healthy young children. There were no patients with AD or SLE who presented with clinical MCV infection. All SLE patients received corticosteroid therapy (mean, 10.0 mg/d of prednisolone). Serum samples from 108 healthy children and adults (48 male and 60 female, aged 0.8 months to 70 years [mean age, 44.4 years]) attending Tokyo University Hospital were used as healthy controls. In addition, we analyzed serum samples from 7 human immunodeficiency virus (HIV)-positive patients with MC (7 male, aged 28-46 years [mean age, 37.5 years]) and 266 HIV-positive patients without MC (242 male and 24 female, aged 22-53 years [mean age, 35.5 years]) attending the Department of Infectious Diseases in Institute of Medical Science, University of Tokyo between 1992 and 1997.

PRODUCTION OF RECOMBINANT VIRUS

Recombinant SeVs were obtained by insertion of an MCV-DNA fragment into a unique NotI site of the SeV transfer plasmid pSeV18b(+), the construction of which was previously described. A truncated ORF sm133 (amino acids 1-398 of MC133L) was generated by polymerase chain reaction with a pair of NotI-tagged primers (5′-CTggcgcgcaAAATCCGTACTATAATTG-3′ and 3′-CTggcgcgcaTTGACCTTCTTTTCTACTACGCGCGTACGGTGCTGCGT GGC-3′). A computer search based on comparison of the amino acid sequence with other known proteins revealed consensus sequences between MC133L and variola virus A30L, a gene encoding 14-kd fusion protein. Although the ORF sm133 and the equivalent region of A30L share 71 evenly distributed amino acids (24% identical), no immunological cross-reactivity has been reported between MCV and variola virus. In addition, no significant amino acid homology has been identified between sm133 and other skin-related viruses (including human herpesviruses and human papillomaviruses), which makes antibody cross-reactivity unlikely. After digestion with NotI, the amplified fragments were directly introduced to the NotI site of pSeV18b(+). This final construct, named pSeV/sm133, gave rise to a full-length copy (16662 base pairs) of the recombinant SeV antigenome. The procedures for transfection and selection of the SeVs were previously described. An allantonic fluid of hen eggs containing 106 pfu/mL of the recombinant and parental viruses was used as the stock viruses for all experiments. CV1 cells were infected with recombinant and parental viruses at the multiplicity of 10 pfu/cell and maintained for 40 hours without serum.

IMUNOBLOTTING

Infected cell lysates and allantonic fluid were electrophoresed in 10% sodium dodecyl sulfate–polyacrylamide gels. The proteins in the gels were electrotransferred onto PVDF membranes (Millipore Co, Bedford, Mass) and probed with 1:20 diluted serum.

ELISA WITH MCV AND RECOMBINANT PROTEINS

ELISA plates (Costar Co, Cambridge, Mass) were coated with 100 mL of allantonic fluid containing 106 pfu/mL of the recombinant and parental SeVs in coating buffer (16-mmol/L sodium carbonate, 44-mmol/L sodium bicarbonate, pH 9.6) at 4°C overnight. A conventional ELISA protocol was used with phosphate-buffered saline containing 0.05% of Tween 20 for washes, 25% dried nonfat milk in phosphate-buffered saline containing 0.05% of Tween 20 (blocking solution) to saturate plates and dilute patient serum (1:350), and a peroxidase-conjugated affinity-purified goat antihuman IgG (Bio-Rad Laboratories, Hercules, Calif.), diluted 1:3000 in blocking solution, followed by a mixture of 3,3′,5′-tetramethylbenzidine and hydrogen peroxidase solution (Bio-Rad). The colorimetric reaction was stopped with 1N sulfuric acid and read spectrophotometrically at 450 nm. All patient serum samples were tested in duplicate, along with 2 positive and 2 negative controls in duplicate. The results were expressed as delta optical density unit (6ODU) (wells coated with allantonic fluid containing recombinant SeV minus wells coated with allantonic fluid containing parental SeV).

STATISTICAL ANALYSIS

The Wilcoxon rank sum test was used to evaluate the significance of differences between the ELISA responses of different groups.

RESULTS

EXPRESSION OF IMMUNOREACTIVE MCV PROTEIN BY RECOMBINANT SeV

In preliminary experiments, about half of the serum samples from patients with MC reacted with recombinant SeV/sm133 protein (Figure 1). CV1 cell lysates and allantonic fluid infected with the recombinant SeV yielded 43-kd immunoreactive polypeptides, which indicated that the truncated MCV protein was secreted in the cell culture medium and present in a solubilized form. The specificity of immunoblotting was demonstrated by the lack of reactivity of 10 serum samples from healthy young children without MC (5 male and 5 female, aged 0.8 months to 1.1 years [mean age, 6.7 months]; data not shown).
Initially, we also expressed a C-terminal hydrophilic domain of MC084L (amino acid 238-298), which showed no reactivity with serum samples from patients with MC. This recombinant protein was not used further.

**DEFINING THE CUTOFF VALUE FOR ELISA WITH RECOMBINANT PROTEIN**

We defined the cutoff for ELISA based on the 12 serum samples from HIV-negative MC patients (7 male and 5 female, aged 2.3-9.4 years [mean age, 5.5 years]) and 10 serum samples from healthy young children without MC. Scrupulous attention was given to avoid false-positive reactions since the allantonic fluid contained some impurities such as SeV and ovalbumin. We found that the mean of absolute ODU could be reduced by less than 0.10 when 10 serum samples from healthy children were applied for the SeV/sm133-based ELISA at dilutions of 1:350 or more. Under this condition, the mean ODU was 0.025 and SD was 0.051. Assuming that these results are indicative of negative responses to the recombinant protein, we established positive antibody as being a value greater than the mean plus 3 SDs (ie, 8ODU of 0.178).

In contrast, 7 of 12 serum samples from young children with MC showed substantial reactivity on ELISA (Figure 2); the mean (SD) 8ODU was 0.330 (0.367).

To verify the validity of the cutoff value, the molecular weight of the immunoreactive protein was visualized by immunoblotting. All of 7 serum samples over the cutoff value reacted both to the SeV/sm133 recombinant protein, and 70-kd protein derived from purified MCV particles by immunoblotting (Figure 1). We also found that another 2 serum samples from infants with MC were negative by SeV/sm133-based ELISA, but were immunoreactive to the 34-kd protein from MCV particles by immunoblotting. The remaining 3 serum samples from infants with MC and 10 serum samples from healthy children without MC did not show any reactivity to recombinant protein or MCV particles.

**SEROPREVALENCE OF ANTIBODIES AGAINST MCV IN A LARGE POPULATION**

Based on the preliminary results described above, all of the serum samples were tested at a dilution of 1:350. Regarding 8ODU as positive at greater than 0.178, we found that 7 (6%) of 108 healthy controls including the 10 young children without MC, 7 (9%) of 76 patients with AD, and 7 (18%) of 39 patients with SLE were seropositive (Figure 2). The reactivity among MC infants was significantly higher than those in the other 3 groups (P<.001). Serum samples from patients with AD and SLE had a higher reactivity than did serum samples from healthy controls (P<.001), while serum samples from HIV-positive patients had a lower reactivity (P<.001). In the HIV-positive group, 1 (14%) of 7 patients with MC and 5 (2%) of 266 patients without MC were seropositive (Figure 2).

We described the first ELISA based on recombinant MCV protein that is useful for serologic diagnosis of MCV infection. It has been shown that the full-length 70-kd protein encoded by MC133L (amino acid 1-546) was potentially inserted into and spanned the cell membrane when the ORF MC133L was expressed in recombinant cowpox virus–infected cells. Furthermore, an immunoelectron microscopic study indicated that the 70-kd protein is localized within the thick layer of the virion. The truncated recombinant protein (amino acid 1-398) was present both in the SeV/sm133-infected cell lysates and culture supernatant (allantonic fluid), which provided evidence that a hydrophobic domain in the N-terminus (amino acid 1-52) contains a functional signal peptide and the other hydrophobic portion near the C-terminus (amino acid 400-466) serves as a unique transmembrane anchoring domain.

Since MCV infection is known to be predisposed by decreased immunity, we investigated the incidence of viral antibodies in patients with AD, SLE, and HIV. A higher ELISA response in AD and SLE may be explained by previous MCV infection. Since it has been reported that MCV is present in apparently normal skin adjacent to an MC lesion in HIV-positive patients, we had expected that a significant antibody production occurred because of subclinical MCV infection. However, the ELISA response among HIV-positive patients without MC was lower than that of the healthy control. Furthermore, only 1 serum sample from 7 HIV-positive patients with MC reacted with the SeV/sm133 antigen, although extensive MC (>20 skin lesions) was observed in all of these patients. Therefore, we considered that the paucity of seroprevalence in HIV-positive patients was ascribed to the impairment of the antibody production system. The situation may be the opposite in patients with hyperimmunity. Epstein et al reported that 8 (11%) of 73 adults without MC were positive and that 50% of the positive reactors had a high serum globulin level associated with internal malignancy. In the present study, 4 (57%) of 7 patients with SLE who were seropositive to MCV showed hypergammaglobulinemia. The possibility exists that other than real clinical or subclinical MCV infection, polyclonal B-cell acti-
vation and hypergammaglobulinemia are associated with a higher ELISA reactivity.

Our results differ from those of another ELISA study for the detection of IgG antibodies against MCV, which found that MCV infection was as common as 23% in the general population, indicating the widespread nature of MCV infection among children and adults. There seem to be some factors that can explain this discrepancy. First, as described above, ELISA reactivity would be affected by the immunological conditions of the host. Konya and Thompson also reported the age-related increase in the prevalence of MCV antibody, which might partly be due to senile hyperglobulinemia. Second, it was possible that our SeV/sm133-based ELISA did not detect sera containing only antibodies to the 34-kd protein of MCV since 2 immunoreactive proteins with a molecular weight of 70 and 34 kd are present within MCV particles. When 12 serum samples from children with MC were examined, 2 of them, which were immunoreactive only to the 34-kd MCV protein by immunoblotting, were not detected by the SeV/sm133-based ELISA. We could not obtain sufficient purified MCV to use as an ELISA antigen, thus it remains unknown how many serum samples containing only antibodies to the 34-kd MCV protein are not detected by the SeV/sm133-based ELISA. Even if the positive predictive value obtained from 12 children with MC (7 of 9) were the same in healthy controls, the proportion of healthy controls who were seropositive for MCV...
would still be lower (6% × 9/7 = 8%) than that reported in the previous study (23%).16 Our overall results indicated that the humoral immune response to MCV is basically confined to patients with MC, and we hypothesized that production of antibodies to MCV does not occur in all patients with clinical MCV infection or that this response is not stable over the course of the infection. This idea is consistent with the fact that 33% (15/46)17 to 59% (32/54)18 of patients with condylomata acuminata are positive in the ELISA assay for papillomavirus, another DNA virus that can grow only in epidermal keratinocytes. Weak immune response to these 2 viruses may be because of the localization of the antigens in the avascular epidermal tissue, which confers protection from the immune system. Recently, 2 proteins encoded by MC053L and MC054L genes of MCV, homologs of mammalian interleukin 18 (IL-18) binding proteins, were shown to bind with high affinity to human IL-18 molecules and inhibited IL-18 mediated interferon-γ production.19 Impairment of interferon-γ production and subsequent T-cell helper type 1 responses due to the presence of viral decoy receptors may provide another mechanism for evasion of the immune system by MCV.

Accepted for publication July 10, 2000.

This work was supported by a grant-in-aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan.

Drs Iwamoto and Tamaki designed and conducted the study. Shioda provided the Sendai virus expression system; and Drs Watanabe, Nakamura, and Wakugawa performed immunoblotting and ELISA; Drs Kato, Nagai, and Shioda provided the Sendai virus expression system; and Drs Iwamoto and Tamaki designed and conducted the study.

Corresponding author and reprints: Takahiro Watanabe, MD, PhD, Dermatology Branch, Bldg 10, Room 12N238, National Cancer Institute, 10 Center Dr, MSC1908, Bethesda, MD 20892-1908 (e-mail: tarr@box-t.nih.gov).

REFERENCES