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Monoclonal Antibodies to the Major Capsid Protein of Human Papillomavirus Type 1

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SUMMARY

Two stable monoclonal hybridoma cell lines secreting type-specific antibodies against the human papillomavirus type 1 (HPV-1) were isolated. The monoclonal antibodies detected HPV-1 antigens in frozen sections of HPV-1-induced warts, using immunofluorescence or immunoperoxidase techniques, and they reacted with HPV-1 particles in an immunodiffusion test. The two monoclonal antibodies recognized the major structural viral polypeptide, with a molecular weight of 54000, and a minor polypeptide, with a molecular weight of 76000, in both the dissociated viral particles and in the wart extracts.

INTRODUCTION

Papillomaviruses constitute a subgroup of the papovaviridae family, and are characterized by the diameter of their naked icosahedral capsid (about 55 nm) and by the size of their genome, a supercoiled circular DNA molecule of about 8000 base pairs (Lancaster & Olson, 1982; zur Hausen, 1981). Viral particles contain a major polypeptide, with a molecular weight in the range of 50000 to 60000, several minor components whose number and origin are unclear and four histone-like polypeptides which are associated with the viral DNA in a minichromosome. (Favre et al., 1975a, 1977; Gissmann et al., 1977; Lancaster & Olson, 1982; Meinke & Meinke, 1981; Orth et al., 1977, 1978; Pass & Maizel, 1973; Pfister et al., 1977). In man, papillomaviruses (PVs) induce benign epithelial tumours of the skin and mucous membranes (warts, condylomas), which may evolve into carcinomas in some instances (Lutzner, 1983; Orth et al., 1980; zur Hausen, 1981). Sixteen types of human papillomaviruses (HPVs) have been identified so far, the basis of classification being a DNA sequence homology on cross-hybridization lower than 50% (Dürst et al., 1983; Lutzner, 1983). The specific association of some HPV types with cutaneous and genital cancers has recently been substantiated (Dürst et al., 1983; Lutzner, 1983; Orth et al., 1980; zur Hausen, 1981). The study of the antigenic properties of the PVs, as well as that of the structural polypeptides of the virions, have been hampered by the unavailability of a system allowing replication in vitro of these viruses (Lancaster & Olson, 1982; Lutzner, 1983; zur Hausen, 1981). Type-specific antigens have been demonstrated by the lack of detectable antigenic cross-reactions between some animal and human PVs and between some skin HPVs (Gissmann et al., 1977; Le Bouvier et al., 1966; Orth et al., 1977, 1980), using hyperimmune sera against intact viral particles. Group-specific antigens, masked in the virions, have been demonstrated, using antisera raised against dissociated PV particles (Jenson et al., 1980; Orth et al., 1978). Hybridoma technology, which allows the isolation of monoclonal antibodies (Köhler & Milstein, 1975), is obviously a method of choice to investigate the antigenic structure of PVs. In that respect, HPV type 1 (HPV-1) constitutes the best model for the study of the PVs infecting humans: large amounts of virus are found in deep plantar warts (Gissmann et al., 1977; Orth et
al., 1977), structural polypeptides of the virions have already been studied (Favre et al., 1975a, 1977; Gissmann et al., 1977; Orth et al., 1977; Pass & Maizel, 1973; Pfister et al., 1977), and the nucleotide sequence of the viral genome has been established (Danos et al., 1983). In this paper, we report the isolation and characterization of two stable hybridoma lines secreting type-specific anti-HPV-1 monoclonal antibodies.

METHODS

Virus. HPV-1 particles were purified from pooled deep plantar warts (Favre et al., 1975a, 1977; Orth et al., 1977). A DNA extract from a sample of the viral suspension gave the cleavage pattern characteristic of HPV-1 DNA, after digestion with a mixture of HindII and HindIII endonucleases (Favre et al., 1975b; Gissmann et al., 1977).

Production of hybridomas and characterization of monoclonal antibodies. Three BALB/c mice received two subcutaneous injections of the virus suspension (50 μg protein) mixed with complete Freund’s adjuvant, with a 2 week interval. Two weeks later, the same amount of viral particles was injected intraperitoneally and the mice were sacrificed after 3 days. Spleen cells (about 2 × 10⁷ cells per animal) were fused with immunoglobulin-non-secreting, 8-azaguanine-resistant SP2/0 myeloma cells (Galfré et al., 1977; Shulman et al., 1978), and hybrid cells were selected as previously described (Roseto et al., 1983). Twelve days later, the media from 400 culture wells were screened for the presence of anti-HPV-1 antibodies. The first test used was the detection of antigens in frozen sections of plantar warts (Orth et al., 1977, 1978) by the indirect immunofluorescence or immunoperoxidase techniques, using anti-mouse IgG sheep immunoglobulins labelled with fluorescein or peroxidase (Institut Pasteur Production, France). The second test was the immunodiffusion technique, using intact viral particles as antigens (Orth et al., 1977, 1978). While the immunohistochemical methods allow the detection of either type-specific or group-specific antigens, the immunodiffusion test reveals mostly type-specific antigens (Orth et al., 1977, 1978).

PAGE, electrophoretic transfer of separated polypeptides from polyacrylamide gels onto nitrocellulose membranes filters and immunoenzymic reactions. Identification of the viral polypeptide(s) bearing the antigenic determinants recognized by the monoclonal antibodies was performed by immune blotting experiments (Towbin et al., 1979). Highly purified HPV-1 virions (Favre et al., 1975a), frozen sections of an HPV-1-induced wart and, as a control, frozen sections of normal human skin, were dissociated by 5 min incubation at 100 °C in the presence of 4 M-urea, 3% SDS and 5% 2-mercaptoethanol (F. Breitburd, O. Croissant, S. Jablonska, J. L. Kienzler & G. Orth, unpublished method). Polypeptides were separated by electrophoresis in a 7.5 to 15% polyacrylamide gradient slab gel in the presence of SDS and transferred electrophoretically to a nitrocellulose membrane (BA85, Schleicher & Schüll) (Burnette, 1981; Shulman et al., 1978). Blots were incubated with the monoclonal antibodies and, as controls, with an anti-HPV-1 type-specific guinea-pig antiserum (G260) (Orth et al., 1977), a group-specific rabbit antiserum against SDS-disrupted HPV-1 particles (R927), and an anti-rotavirus monoclonal antibody (160C19) (Roseto et al., 1983).

RESULTS AND DISCUSSION

Two hybridoma cultures (B6 and D5) were found positive by both tests employed, suggesting that they secreted type-specific antibodies. Cultures were cloned by dilution and two clones (334B6 and 405D5) were subcultured and further characterized. The secretion of antibodies was still observed after 12 months, and titres, expressed as the reciprocal of the highest dilution giving a strong staining of infected nuclei in sections (Fig. 1a), or a precipitation line with viral particles, varied between 10 to 100 or 1 to 16, respectively. Ascitic tumours were induced in BALB/c mice by intraperitoneal injection of 2 × 10⁶ hybridoma cells. The antibody titres of ascitic fluids obtained 12 to 18 days later ranged from 200 to 400, as evaluated by immunofluorescence and immunoperoxidase tests (Fig. 1b), or from 50 to 200, as assayed by immunodiffusion (Fig. 2).

The identification of the class of immunoglobulins secreted by the hybridomas was performed by an immunodiffusion test, using rabbit antibodies to mouse immunoglobulins (Nordic Immunological Laboratories, Maidenhead, U.K.). The hybridoma cell clones 334B6 and 405D5 secreted antibodies of the IgG2a subclass and the IgG1 class, respectively.

The two monoclonal antibodies and a polyclonal guinea-pig antiserum raised against intact HPV-1 virions (G260) (Orth et al., 1977) gave a continuous precipitation line, when tested against HPV-1 particles by the immunodiffusion method (Fig. 2).

While the two monoclonals detected HPV-1 antigens in frozen sections of plantar warts at a 1/400 dilution, no reaction was observed at a 1/10 dilution using frozen sections of warts induced by HPV types 2, 3, 4, 5 or 7 (Lutzner, 1983), neither by the immunofluorescence nor the immuno-
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Fig. 1. Detection of HPV-1 antigens in sections of plantar warts. Frozen wart sections (Orth et al., 1977, 1978) were incubated with (a) purified immunoglobulins, used at 0.1 mg/ml, obtained from the supernatant of a 334B6 cell culture after ammonium sulphate precipitation and Sephadex G-200 gel filtration (Orth et al., 1978), or (b) 405D5 ascitic fluid at 1/200 dilution. Immune complexes were detected using anti-mouse IgG sheep immunoglobulins labelled either with fluorescein (a) or peroxidase (b). Peroxidase activity was revealed by incubation of the sections in the presence of H₂O₂ and 3'-diaminobenzidine. Viral antigens were detected in the nuclei of keratinizing cells and in keratinized cells. × 220.

Fig. 2. Precipitation of HPV-1 virions by monoclonal antibodies. The immunodiffusion test was performed in a 0-5% agarose gel (Orth et al., 1977, 1978), using purified HPV-1 virions (10 µg protein) (central well), 334B6 ascitic fluid at 1/40 dilution (a), 405D5 ascitic fluid at 1/40 dilution (b), anti-HPV-1 antiserum (G260) at 1/40 dilution (c, f) or phosphate-buffered saline (d, e). Diffusion was allowed to proceed for 24 h at 25 °C.

peroxidase methods (data not shown). Similarly, no staining was observed using sections of Bouin's fixed, paraffin-embedded HPV-1-induced wart, where the reactivity of type-specific antigens is considerably diminished or abolished, in contrast to group-specific antigens (Jenson et al., 1980).

Taken together, these results demonstrate that the antibodies secreted by the 334B6 and 405D5 cell lines were type-specific.
Fig. 3. Identification of the HPV-1 polypeptides detected by the monoclonal antibodies. Immune blotting experiments were performed in conditions previously reported (Roseto et al., 1983; Towbin et al., 1979; F. Breitburd et al., unpublished methods). Polypeptides from SDS-disrupted HPV-1 virions (2.5 µg protein) (a, d, g, j, m), human skin sections (40 µg protein) (b, e, h, k, n) and HPV-1-induced plantar wart sections (40 µg protein) (c, f, i, l, o) were separated by electrophoresis in the presence of SDS and transferred onto a nitrocellulose membrane. Electrophoretic migration and transfer were checked by staining a gel with Coomassie Brilliant Blue (a to c) and a blot with amido black (d to f). After saturation in the presence of 3% bovine serum albumin, blots were incubated with an antiserum against SDS-disrupted HPV-1 particles (R927) at 1/100 dilution (g to i), and with 334B6 (j to l) or 405D5 (m to o) purified immunoglobulins at 0.2 mg/ml. After washing, membranes were incubated either with peroxidase-labelled protein A (g to i) or peroxidase-labelled anti-mouse IgG sheep immunoglobulins (j to o). Peroxidase activity was detected in the presence of H2O2 and 3'-diaminobenzidine. Mol. wt. (× 10^-3) of the polypeptide constituents of the virions are indicated.

The viral constituents observed on the Coomassie Brilliant Blue-stained gels (Fig. 3 a to c) and on the amido black-stained blots (Fig. 3 d to f) were of a major polypeptide with an apparent molecular weight of 54000, a minor polypeptide with an apparent molecular weight of 76000 and the histone-like polypeptides with apparent molecular weights ranging from 12500 to 16500 (Fig. 3 a, d), in agreement with previously reported results (Favre et al., 1975a, 1977; Orth et al., 1977, 1978). Immune complexes were detected by incubation with peroxidase-labelled anti-mouse IgG sheep immunoglobulins or peroxidase-labelled Protein A (F. Breitburd et al., unpublished methods). Polyclonal antibodies raised against intact HPV-1 virions (data not shown) or against disrupted HPV-1 particles and the two monoclonal antibodies reacted with the 54000 and the 76000 polypeptides of the dissociated viral particles (Fig. 3g, j, m). The polyclonal antibodies also detected these two polypeptides in the wart extracts (Fig. 3i). In these conditions, the monoclonals revealed the 54000 polypeptide but the reaction with the 76000 species was at the limit of detection (Fig. 3l, o). The polyclonal and monoclonal antibodies revealed species with molecular weights greater than 100000 and in the range of 40000 to 50000 (Fig. 3g, i, j), which were barely or not detected on the stained gels or blots of the disrupted viral particles. These minor species could constitute incomplete disruption products of the virions or degradation products of the major viral polypeptide, as previously discussed (Favre et al.,...
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1975a; Gissmann et al., 1977; Lancaster & Olson, 1982; Meinke & Meinke, 1981; Pfister et al., 1977). The anti-rotavirus antibodies showed no reaction with the HPV-1 polypeptides (data not shown) and the polyclonal or monoclonal antibodies detected neither a 76000 nor a 54000 polypeptide in the normal human skin extracts (Fig. 3 h, k, n). Some staining was irregularly observed with polypeptides migrating like the keratins or the H1, H3 and H4 histones.

These immune blotting experiments support the conclusion that the 76000 mol. wt. polypeptide, reproducibly detected in our previous studies (Favre et al., 1977; Orth et al., 1977, 1978), is most likely a structural component related to the major 54000 polypeptide. The region of the HPV-1 genome assumed to be expressed only in productive infection contains two open reading frames, L1 and L2, with a coding capacity for a 57000 and a 55000 polypeptide, respectively (Danos et al., 1983; Engel et al., 1983). One of these two genes could encode the 54000 species. As for the 76000 polypeptide, it has to be assumed that it is translated from a spliced messenger RNA containing sequences of the gene coding for the 54000 polypeptide. Nucleotide sequences conserved among PVs are found in L1 and at the 5' end of L2 (Danos et al., 1983), which may account for the existence of the PV group-specific antigen(s) (Jenson et al., 1980; Orth et al., 1978). The isolation of hybridoma cell lines secreting antibodies with a group specificity should allow progress in the characterization of the virus-coded polypeptides.

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REFERENCES


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