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A synthetic peptide defines a serologic IgA response to a human papillomavirus-encoded nuclear antigen expressed in virus-carrying cervical neoplasia

(immunoglobulin A/viral transformation/tumor detection/ELISA)

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ABSTRACT **The growing awareness of the role of human papillomavirus (HPV) in cervical carcinoma has triggered a search for uncomplicated detection methods. To define a serologic response to HPV, we synthesized peptides based on sequences deduced from the genome of HPV type 16, the most common malignancy-associated type of HPV. One of these peptides reacted with IgA antibodies present in sera from 24 of 33 patients with cervical intraepithelial neoplasia or cervical carcinoma, whereas this peptide reacted with only 6 of 27 sera from individuals without cervical intraepithelial neoplasia. Immunoaffinity-purified human antipeptide IgA antibodies detected HPV-specific 58- and 48-kDa proteins in cervical carcinoma cell extracts and also detected a nuclear antigen in HPV-carrying cervical cancer cell lines and cervical intraepithelial neoplasia biopsied tissue. These antigens were also detected with mouse monoclonal and rabbit polyclonal antibodies to the same peptide. The results indicate that screening for infection with malignancy-associated types of HPV may be possible by simple synthetic peptide-based serology.**

The human papillomavirus (HPV) exists as at least 56 different types (1). Diseases associated with particular types differ—e.g., HPV types 1 and 2 cause plantar and common warts, types 6 and 11 cause benign genital warts (condylomas), whereas types 16, 18, 31, 33, and 35 are found in HPV-carrying cervical intraepithelial neoplasia (CIN) (1, 2) as well as in most cervical carcinomas, suggesting an etiologic relationship of these types of HPV to cervical cancer (1, 3, 4).

DNA sequencing of several of the HPVs has defined open reading frames (ORFs), numbered *L1*, *L2*, and *E1–E7*, on one strand of the viral DNA (1). *L1*, *L2*, and *E4* encode late proteins present in virus-producing tissue (5–7). E6 and E7 proteins can be immunoprecipitated from certain cervical cancer cell lines (8, 9). The *E2* gene encodes a transactivating factor (10), and an E2 protein has been detected on immunoblots of condylomas (11). Two of 72 condyloma patients had IgG antibodies against an E2-derived fusion protein (12).

The HPV capsid antigen is regularly immunogenic (5, 13) for both IgA and IgG antibodies (14) and is present in the outer layer of the infected epithelium (1, 13). In contrast, in the basal-cell layer HPV genomes can be demonstrated (1), but no viral antigen has been found (1, 13). Similarly, viral-capsid antigen is frequently detected in condylomas, but this antigen is infrequent or absent in the carcinomas (1, 13). Although several HPV proteins have been found in extracts from tumor biopsies and cell lines, whether HPV encodes tumor antigens expressed in all these HPV-infected cells is unknown. Because the other human DNA tumor viruses, Epstein–Barr virus (EBV) and hepatitis B virus, express such tumor antigens (15, 16), the existence of analogous HPV tumor antigens is very likely.

A relevant phenomenon in tumor-virus serology is the IgA response to EBV. Although EBV is continuously present in many healthy individuals, measuring IgA antibodies to EBV is a highly specific and sensitive test for early detection of the EBV-associated nasopharyngeal carcinoma (17). The reason for the tumor specificity of the EBV IgA response is not known, but presumably this specificity involves an inflammatory reaction of mucous membranes caused by the growing tumor.

In recent years, the use of serology for diagnosis of viral diseases and tumors has been vastly simplified by the invention of site-directed serology with synthetic peptide-based ELISA. We have previously shown the usefulness of this method in several systems—e.g., for the diagnosis of infection with EBV (18), respiratory syncytial virus (19), the human immunodeficiency viruses (20, 21), and of hepatitis B-associated hepatocellular carcinoma (16).

We therefore wanted to address the following questions: Can HPV-encoded tumor antigens be demonstrated? Is there an antigenic response to such antigens in patients with cervical neoplasia? In particular, is there an IgA response? If so, can an exact epitope be defined? Can cervical neoplasia be detected by immunostaining of HPV-16 tumor antigens or by simple measurement of antibodies to HPV-16-derived synthetic peptides in patient sera? In this report, we show that there is a cervical neoplasia-associated IgA response to an HPV-derived synthetic peptide and that corresponding antipeptide antibodies detect an HPV-encoded tumor antigen in HPV-carrying cervical carcinoma cells.

MATERIALS AND METHODS

Peptide Synthesis. Seven 11- to 19-residue peptides were synthesized by the solid-phase method, as described (22). Peptides were analyzed by amino acid analysis and for purity by HPLC by use of a reverse-phase Vydac C_4 column.

ELISA. The synthetic peptides at 20 μg/ml in 10 mM sodium carbonate buffer, pH 9.3, were coated onto ELISA plates overnight at room temperature. Blocking of the plates with 10% horse serum/phosphate buffered saline (HS/PBS) for 1 hr at 37°C followed, after which the patient sera were added in duplicates at a 1:20 dilution in HS/PBS and incu-

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Abbreviations: HPV, human papillomavirus; CIN, cervical intraepithelial neoplasia; mAb, monoclonal antibody; EBV, Epstein–Barr virus; PBS, phosphate-buffered saline; ORF, open reading frame.

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bated for 2 hr at 37°C. After washing of the plates with PBS/0.05% Tween 20, peroxidase-conjugated monoclonal antihuman IgA (Janssen) was added at a 1:1000 dilution in HS/PBS and incubated as above. The plates were washed and developed with 2,2'-azinobis(3-ethylbenzthiazolinium-6-sulfonate) at 0.4 mg/ml/0.01% H_2O_2/0.1 M citrate buffer, pH 4, for 60 min before recording *A* values at 405 nm.

Affinity Purification of Antipeptide Antibodies. Ten milligrams of the selected peptide His-Lys-Ser-Ala-Ile-Val-Thr-Leu-Thr-Tyr-Asp-Ser-Glu-Trp-Gln-Arg-Asp-Gln-Cys (no. 245) was coupled to 4-ml CH Sepharose 4B (Pharmacia), as recommended by the manufacturer. After pre-elution with 4 M KSCN, the column was equilibrated with 400 ml of PBS. Two milliliters of human serum or 5 ml of rabbit serum were applied at 5 ml/hr first to a CH Sepharose 4B column containing an irrelevant peptide and then to the peptide-245 column. Thereafter, the peptide-245 column was washed with PBS/0.5 M NaCl for 20 column vol or until no protein was detectable in the effluent. Bound antibodies were eluted with 4 M KSCN and dialyzed extensively against PBS.

Immunoblotting. Ten milligrams of cultured cells were dissolved in 100 μl of electrophoresis sample buffer (23), electrophoresed on 7% polyacrylamide gels, and transferred to nitrocellulose sheets (24). After being blocked in 5% milk for 1 hr and incubated with antipeptide antibody in 0.5% milk for 12 hr, the sheets were washed in 5% milk, incubated with alkaline phosphatase-conjugated anti-human IgA, anti-mouse IgG, or anti-rabbit IgG antibodies (Sigma) diluted 1:1000. After four 30-min washings in PBS/0.05% Tween 20, the blots were developed with nitro blue tetrazolium.

Immunohistocytochemistry. Cell lines were grown on slides, washed with 100 mM NaCl, and fixed in acetone at −20°C for 20 min. Biopsies were snap-frozen and 4-μm sections were cut. The slides were rehydrated in PBS, incubated with 3% (vol/vol) H_2O_2 for 15 min, with 10% horse serum for 30 min, and with the hybridoma supernatant containing the monoclonal antibody (mAb) designated 245:11E3, diluted 1:18 in 0.5% milk, or with affinity-purified human antipeptide-245 antibodies, diluted 1:5, for 90 min. Between PBS washes, the slides were further incubated with biotinylated horse anti-mouse IgG (Vector Laboratories) at 10 μg/ml in HS/PBS for 45 min and then with avidinD–peroxidase (vector) at 10 μg/ml in PBS for 30 min and 0.025% 3-amino-9-ethylcarbazole/0.012% H_2O_2 in sodium acetate, pH 5, for 30 min.

Additional Technical Information. The CIN biopsies used for immunostaining were from the Georgetown University Medical Center, Washington, DC; HPV-carrier status of this material was determined by Southern blotting (25). All patient sera were from the Green Hospital of Scripps Clinic, La Jolla, CA. Pathological lesions of the corresponding cervical biopsies were diagnosed according to Meisels and Fortin (2). Cervical carcinoma cell lines were obtained from the American Type Culture Collection (ATCC) and cultured according to ATCC specifications. HPV-16-carrying NIH 3T3 cells were the gift of J. A. DiPaolo (National Cancer Institute) (26). Synthetic peptides were coupled to keyhole limpet hemocyanin (27) and used for immunization of 129GIX$^+$ mice or New Zealand White rabbits, as described (28, 29). The procedure for isolation of mAbs has been reported (28).

RESULTS

We synthesized seven peptides based on hydrophilic, conserved amino acid sequences deduced from selected ORFs of the HPV-16 genome. The peptides were tested for antigenicity by ELISA against human sera from 33 patients with CIN or cervical carcinoma as compared with human sera from 27 donors not known to have CIN. One of these peptides (no. 245) was deduced from a region close to the carboxyl terminus of the *E2* ORF and had the sequence His-Lys-Ser-Ala-Ile-Val-Thr-Leu-Thr-Tyr-Asp-Ser-Glu-Trp-Gln-Arg-Asp-Gln-Cys. This peptide reacted with IgA antibodies in the sera of 24 of 33 patients with CIN or cervical carcinoma but reacted with only 6 of 27 sera from donors not known to have CIN (Fig. 1). The difference in ELISA *A* values between the two groups was significant, $P < 0.002$ (Mann–Whitney test). There was also a difference in IgG reactivity between the two groups, but since this difference was much less significant ($P < 0.005$), the IgG antibodies were not further studied.

The two CIN patient sera most reactive with peptide-245 were applied to columns of peptide-245 bound to Sepharose. After washing and elution of the column, the eluted peptide-specific antibodies were applied to immunoblots of extracts from the HPV-16-carrying cervical carcinoma cell line CaSki (30), the HPV-18-carrying cervical carcinoma cell line C-4 II (30), and NIH 3T3 cells stably transfected with the complete HPV-16 genome (26). As controls, we used the HPV-negative cervical carcinoma cell lines HT-3 and C-33A (30), the cervical carcinoma cell line SiHa that carries a single genome of HPV-16, interrupted in the *E2* ORF (31), and untransfected NIH 3T3 cells (26). The antipeptide antibodies from one patient detected a 48-kDa protein in the C-4 II cells and 48- and 26-kDa proteins in the HPV-16-transfected NIH 3T3 cells; no reaction was seen in any control cells (Fig. 2*A*). In CaSki cells, only a weak reaction with a 58-kDa protein was detected (Fig. 2*A*, second column). The antipeptide antibodies from the other serum also reacted with the same 58-kDa and 48-kDa proteins (data not shown).

To characterize these proteins further, we produced a mouse mAb and rabbit antiserum against peptide-245. The mAb, designated 245:11E3, reacted with the 58-kDa protein present in CaSki cells, but not in HT-3 or SiHa control cells (Fig. 2*B*). However, the affinity-purified rabbit antipeptide antibodies reacted mainly with a 48-kDa protein of CaSki cells (Fig. 2*C*), minimally with 51- and 58-kDa CaSki proteins, but not with the control cell lines.

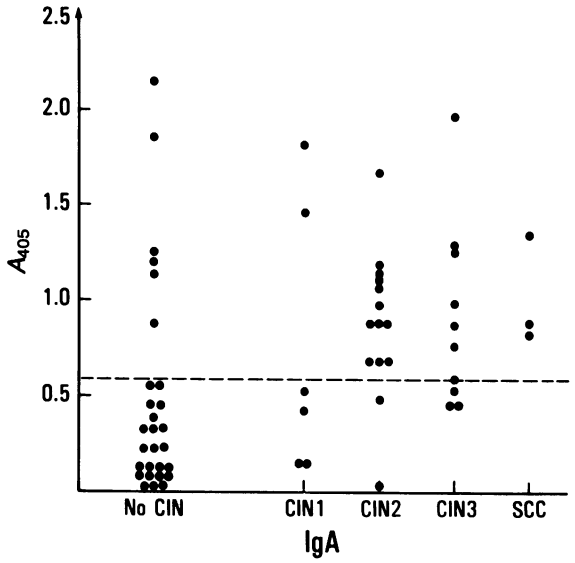

FIG. 1. ELISA for detection of antipeptide IgA antibodies in sera from CIN patients. No CIN, 27 serum donors not known to have CIN; in this group, 10 donors were healthy, 6 donors had condyloma, and 11 donors had diseases unrelated to HPV. No significant differences in reactivity among these subgroups were noted. CIN1, CIN2, and CIN3 represent mild dysplasia, moderate dysplasia, and carcinoma *in situ*, respectively (2). SCC, invasive squamous cell carcinoma. Each value represents mean of duplicate *A* values after subtraction of the mean of a duplicate blank (no peptide added to plate). An *A* value of 0.6 (three times background) was considered significant.

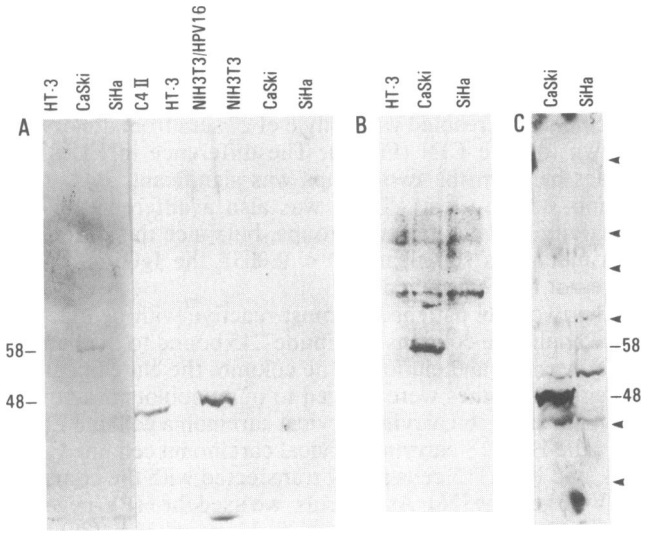

FIG. 2. Detection of HPV proteins by immunoblotting with antipeptide antibodies. (*A*) Antipeptide-245 IgA antibodies immunoaffinity purified from the serum of a patient with *in situ* carcinoma, final dilution 1:10. (*B*) Antipeptide-245 mouse mAb 245:11E3 in undiluted hybridoma supernatant. (*C*) Immunoaffinity-purified rabbit antipeptide-245 antibodies, final dilution 1:32. Two additional CaSki-specific bands of 51 and 58 kDa were seen on overexposure. Several weak reactivities not specific for CaSki are seen in *B* and *C*. These reactivities were also seen on control blots with irrelevant mouse and rabbit antipeptide antibodies. Figures denote molecular mass in kDa; arrowheads indicate positions of the molecular mass markers (200, 116, 92, 66, 44, and 31 kDa, respectively). CaSki, HPV-16-carrying cervical carcinoma cell line; C-4 II, HPV-18-carrying cervical carcinoma cell line; SiHa, cervical carcinoma cell line carrying a single genome of HPV-16, which has been interrupted in the *E2* ORF; HT-3, HPV-negative cervical carcinoma cell line. NIH 3T3/HPV-16, mouse fibroblast NIH 3T3 cells, stably transfected with HPV-16 (26). The left three columns of *A* and all columns of *B* were developed for 12 hr, whereas the right columns of *A* and both columns of *C* were developed for 30 min.

To investigate whether all cells of the HPV-carrying cancer cell line expressed the detected proteins, we immunostained acetone-fixed CaSki cells, in parallel with the C-33A, HT-3, and SiHa control cells. mAb 245:11E3 and the human peptide-specific IgA antibodies stained all cells in the CaSki cell line; strong staining was seen in the nucleus, but some cytoplasmic staining was also seen (Fig. 3*A*). Control cells stained only faintly and only in the cytoplasm (Fig. 3*B*). Addition of free peptide-245 at 1 mg/ml blocked all reactivity, but addition of control peptide had no effect. We also performed subcellular fractionation (32) of CaSki cells and immunoblotted the fractions with mAb 245:11E3. The 58-kDa protein was found exclusively in the nuclei, not at all in the cytoplasmic or cell membrane/microsomal fractions (data not shown).

To establish whether all HPV-infected cells also express the 58-kDa antigen *in vivo*, we immunostained sections of 14 HPV-16-carrying, 2 HPV-31-carrying, and 2 HPV-11-carrying cervical and vaginal intraepithelial neoplasias, as well as sections from the normal cervix of a 6-yr-old female. mAb 245:11E3 stained all the HPV-16- and HPV-31-positive neoplasias, but not the HPV-11-carrying neoplasias or the 6-yr-old female's cervical section (shown in Fig. 3 *C* and *D*). The staining extended throughout the epithelium down to the basal-cell layer (Fig. 3*C*) and decreased gradually in intensity in the areas adjacent to the pathological lesion.

DISCUSSION

We have used a synthetic peptide to define a regularly immunogenic epitope in the *E2* ORF of HPV-16. The HPV-

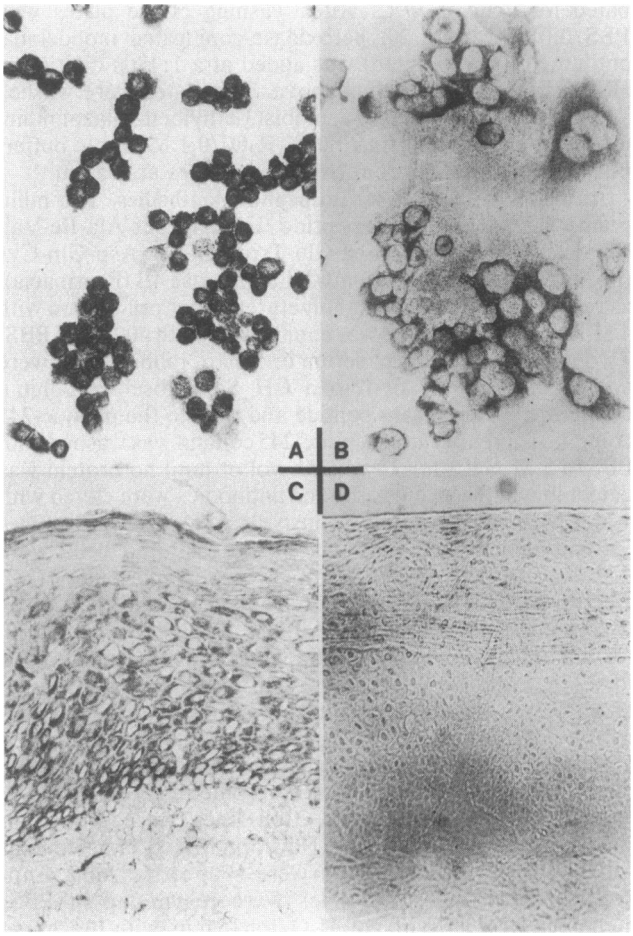

FIG. 3. Detection of an HPV nuclear antigen by immunostaining. Mouse antipeptide mAb 245:11E3 was used for staining HPV-16-carrying CaSki cells (*A*) and HPV-negative C-33A cells (*B*); the additional control cell lines SiHa, HT-3, and HeLa were also negative. Stainings with human antipeptide IgA antibodies were similar to those obtained with the mAb. Control staining of CaSki cells with irrelevant antibodies and with antipeptide antibodies blocked with free peptide at 1 mg/ml yielded results similar to those in *B*. (*C*) An HPC-16-carrying CIN biopsy stained with mAb 245:11E3. (*D*) Similarly stained biopsy from the cervix of a 6-yr-old female. (*A–B* ×150; *C–D* ×75.)

specificity of the human anti-peptide antibodies was shown both by their correlation to the diagnosis of CIN ($P < 0.002$) and by the finding that the purified human antipeptide antibodies were reactive with HPV-specific antigens in immunoblotting and immunostaining. Four distinct HPV-specific proteins of 26, 48, 51, and 58 kDa were identified; the 48-kDa species appeared the most abundant of these proteins. In the CaSki cell line, the 48-kDa protein was only detected by the rabbit antipeptide antibodies, suggesting that the particular HPV-16 strain (30) carried by these cells may have a sequence variation in the peptide-defined epitope. The distinct molecular mass species are probably generated by different splicing patterns (33).

The fact that two long-term established cervical carcinoma cell lines have retained expression of these proteins suggests that interruption of the *E2* ORF is not important for HPV-induced transformation. Rather, the immunostaining results with both the mouse mAb and the human antipeptide antibody suggest that the nuclear antigen may, in fact, be a candidate for a viral-transforming protein: the detected antigen was constitutively expressed throughout both cell lines and infected epithelium, indicating that the antigen is an HPV tumor antigen, rather than part of an early, transitory phase

in the virus replicative cycle. In the latter case, the antigen would have been expressed only in some cells in the cell lines and some layers of the infected epithelium. Thus, the use of our mAb should enable immunostaining-based detection of nonpermissive HPV infection.

Furthermore, the reaction of the mAb with HPV-16 and HPV-31-carrying CIN lesions, but not with HPV-11-carrying lesions, suggests that both detection and typing of latent HPV infection are possible by simple immunohistocytochemistry. Although most staining was found in the nuclei, a significant amount of cytoplasm stained, especially in the biopsies. This result contrasted with our finding that the 58-kDa polypeptide detected by immunoblotting was found exclusively in the nucleus by subcellular fractionation. A possible explanation is that antipeptide antibodies can sometimes react with small fragments of the intact protein, present in the cytoplasm as part of normal protein turnover (34). Note also that we cannot determine which of the distinct molecular mass species seen by immunoblotting corresponds to the antigen seen by immunostaining.

The most significant result is our detection of a serological reaction to an HPV nuclear antigen, constitutively expressed in some cervical cancers. This detection could form the basis for simple monitoring of the enormous population at risk for HPV-induced genital tract neoplasia, as we identified approximately three-quarters of such patients by using an ELISA in which IgA antibodies in the sera of these patients bound synthetic peptide-245 of HPV-16. The remaining one-fourth of CIN patients may have been uninfected or infected with HPV types not cross-reactive with HPV-16. Comparison with other HPVs of the sequences corresponding to peptide-245 showed that cross-reactions may exist with HPV-6, -11, -18, and -33, but determination of which HPV types the present ELISA does measure awaits further experiments. Possibly the CIN detection rate could be improved if patient sera were also tested against homologous peptides deduced from the other malignancy-associated types of HPV.

Although a rather large percent (22%) of serum donors without known CIN were positive in the IgA ELISA, the cause may, in some cases, have been undiagnosed CIN. A recent study has suggested that HPV-16 genomes are present in more than one-fourth of apparently normal cervical biopsies (35). HPV-16 can also be carried by oral warts and can be transmitted from mother to child during parturition (36). Therefore, obtaining a control group of definitely uninfected patients is difficult. Even though a few sera may have been false positives, a positive ELISA result can be unambiguously confirmed by immunoblotting, as shown here. These inexpensive immunological methods could have important application in the identification and epidemiologic study of women with HPV infection who may be at risk for cervical carcinoma.

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1. Pfister, H. (1987) *Adv. Cancer Res.* **48,** 113–147.
2. Meisels, A. & Fortin, R. (1976) *Acta Cytol.* **20,** 505–509.
3. Zur Hausen, H. (1977) *Curr. Top. Microbiol. Immunol.* **78,** 1–30.
4. Syrjanen, K. J. (1984) *Obstet. Gynecol. Surv.* **39,** 252–265.
5. Li, C. C. H., Shah, K, V., Seth, A. & Gilden, R. V. (1987) *J. Virol.* **61,** 2684–2690.
6. Komly, C. A., Breitburd, F., Croissant, O. & Streeck, R. E. (1986) *J. Virol.* **60,** 813–816.
7. Doorbar, J., Campbell, D., Grand, R. & Gallimore, P. (1986) *EMBO J.* **5,** 355–362.
8. Smotkin, D. & Wettstein, F. O. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 4680–4684.
9. Seedorf, K., Oltersdorf, T., Krammer, G. & Rowekamp, W. (1987) *EMBO J.* **6,** 139–144.
10. Phelps, W. C. & Howley, P. M. (1987) *J. Virol.* **61,** 1630–1638.
11. Li, C. C. H., Gilden, R. V., Showalter, S. D. & Shah, K. V. (1988) *J. Virol.* **62,** 606–609.
12. Jenison, S. A., Firzlaff, J. M., Langenberg, A. & Galloway, D. A. (1988) *J. Virol.* **62,** 2115–2123.
13. Walker, P. G., Singer, A., Dyson, J. L., Shah, K. V., To, A. & Coleman, D. V. (1983) *Br. J. Cancer* **48,** 99–101.
14. Dillner, L., Bekassy, Z., Jonsson, N., Moreno-Lopez, J. & Blomberg, J. (1989) *Int. J. Cancer* **43,** 36–40.
15. Klein, G., Giovanella, B. C., Lindahl, T., Fialkow, P. J., Singh, S. & Stehlin, J. S. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 4737–4741.
16. Moriarty, A. M., Alexander, H., Lerner, R. A. & Thornton, G. B. (1985) *Science* **227,** 429–433.
17. Henle, G. & Henle, W. (1976) *Int. J. Cancer* **17,** 1–7.
18. Dillner, J., Sternas, L., Kallin, B., Alexander, H., Ehlin-Henriksson, B., Jornvall, H., Klein, G. & Lerner, R. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 4652–4656.
19. Norrby, E., Mufson, M. A., Alexander, H., Houghten, R. A. & Lerner, R. A. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 6572–6576.
20. Smith, R. S., Naso, R. B., Rosen, J., Whalley, A., Hom, Y. L., Hoey, K., Kennedy, C. J., McCutchan, J. A., Spector, S. A. & Richman, D. D. (1987) *J. Clin. Microbiol.* **25,** 1498–1504.
21. Norrby, E., Biberfeld, G., Chiodi, F., von Gegerfeldt, A., Naucler, A., Parks, E. & Lerner, R. (1987) *Nature (London)* **329,** 248–250.
22. Houghten, R. A. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 5131–5135.
23. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
24. Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4350–4354.
25. Southern, P. J. & Berg, P. (1982) *J. Mol. Appl. Genet.* **1,** 327–341.
26. Yasumoto, S., Burkhardt, A. L., Doniger, J. & DiPaolo, J. A. (1986) *J. Virol.* **57,** 572–577.
27. Liu, F. T., Zinnecker, M., Hamaoka, T. & Katz, D. H. (1979) *Biochemistry* **18,** 690–697.
28. Dillner, J., Wendel-Hansen, V., Kjellstrom, G., Kallin, B. & Rosen, A. (1988) *Int. J. Cancer* **42,** 721–727.
29. Sutcliffe, J. G., Shinnick, T. M., Green, N., Lin, F. T., Niman, H. L. & Lerner, R. A. (1980) *Nature (London)* **287,** 801–807.
30. Yee, C., Krishnan-Hewlett, I., Baker, C., Schlegel, R. & Howley, P. (1985) *Am. J. Pathol.* **119,** 361–366.
31. Baker, C. C., Phelps, W. C., Lindgren, V., Braun, M. J., Gonda, M. A. & Howley, P. M. (1987) *J. Virol.* **61,** 962–971.
32. Jett, M., Seed, T. M. & Jamieson, G. A. (1977) *J. Biol. Chem.* **252,** 2134–2142.
33. Petterson, U., Ahola, H., Stenlund, A. & Moreno-Lopez, J. (1987) in *The Papovaviridae*, eds. Salzman, N. P. & Howley, P. M. (Plenum, New York), Vol. 2, pp. 67–108.
34. Hatzubai, A., Anafi, M., Masucci, M. G., Dillner, J., Lerner, R. A., Klein, G. & Sulitzeanu, D. (1987) *Int. J. Cancer* **40,** 358–364.
35. Cox, M. F., Meanwell, C. A., Maitland, N. J., Blackledge, G., Scully, C. & Jordan, J. A. (1986) *Lancet* **ii,** 157–158.
36. Shah, K. V. & Buscema, J. (1988) *Annu. Rev. Med.* **39,** 371–379.