PAPILLOMA VIRUSES: MOLECULAR AND CLINICAL ASPECTS

Peter M. Howley and Thomas R. Broker, Organizers

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Papilloma Viruses and Human Genital Tract Diseases

1307 DETECTION OF HPV DNA IN HUMAN GENITAL LESIONS, Lutz Gissmann, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, 6900 Heidelberg, FRG

Since it has been shown that particular human papillomavirus types (HPV16 and 18) are preferentially associated with malignant genital tumors whereas others (HPV6, 11) are mainly found in benign lesions. determination of the HPV type might be of diagnostic relevance in order to elucidate the biological potential of single genital lesions to develop into a malignant tumor (1, 2).
Analysing the molecular state of HPV DNA it has been shown that integration into the host genome might be an important step for malignant transformation since the viral molecules permit exclusively an extrachromosomal circle within the benign lesions.

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2. Wagner, D., Ikenberg, H., Böhm, N., and Gissmann, L. (1985): Identification of Human Papillomavirus in Cervical Smears by Deoxiribonucleic Acid in Situ Hybridization. Obst. Gynecol. 65 (in press).

1308 PAPILLOMAVIRUSES AND CERVICAL NEOPLASIA: REFLECTIONS ON THE PAST, PERCEPTIONS OF THE PRESENT, AND SPECULATIONS ON THE FUTURE. R.J. Kurman, A.B. Jenson, and W.D. Lancaster. Georgetown Univ. Med. Ctr., Washington, DC 20007

The recognition that human papillomaviruses (PV) are associated with benign genital warts (condyloma acuminata) and precancerous lesions of the cervix (dysplasia, carcinoma-in-situ (CIS) or alternatively cervical intraepithelial neoplasia (CIN) has raised a number of questions concerning the etiology, pathologic diagnosis, and clinical management of patients with cervical dysplasia. Traditionally, cervical dysplasia was regarded as a precancerous lesion although it was recognized that a high proportion of these lesions spontaneously regressed or persisted and only a minority progressed to invasive cancer. Moreover, the behavior of a particular dysplastic lesion based on its histologic appearance has been notoriously unpredictable. In view of its capricious behavior the clinical management of women with dysplasia has ranged from observation to hysterectomy.

The evidence relating PV to cervical neoplasia is based on morphologic, immunocytochemical and molecular virologic data. Thus, CIN is composed of a group of lesions which form a morphologic continuum ranging from mild to severe dysplasia that merges imperceptibly with CIS. PV-specific structural antigens and DNA sequences have been identified within all phases of the intraepithelial process leading up to CIS (1,2,3). A number of different PVs including HPV-16 have been detected in cervical dysplasias (4) and HPV-16 has been identified in a high proportion of invasive cervical cancers (5). These data provide further evidence implicating PV in the genesis of cervical neoplasia and also suggest that cervical abnormalaties traditionally classified as "dysplasia" may be composed of a heterogeneous group of lesions that are induced by a limited number of PV types of varying oncogenic potential. Perhaps only a subset of PV types acting in concert with other factors i.e., herpes infection, specific defects in host cell mediated immunity etc. induce neoplastic transformation whereas the majority produce lesions with little or no oncogenic potential that persist as chronic infections or regress spontaneously.

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1309 NATURAL HISTORY OF HPV INFECTIONS IN UTERINE CERVIX AS DETERMINED BY PROSPECTIVE FOLLOW-UP, Kari J. Syrjänen, Martti Väyrynen, Rauno Mäntyjärvi, Seppo Saarikoski, and Olli Castrén, Department of Pathology, Gynecology&Obstetric, and Clinical Microbiology, University of Kuopio, SF-70211 Kuopio, Finland

To assess the natural history of cervical HPV lesions, i.e. flat, inverted and papillomatous condylomas, a long-term prospective follow-up study was started in October 1981. At this writing, a total of 343 women have been followed-up for a mean of 18 15 (M SD) months, either by cervical punch biopsy or Papanicolaou (PAP) smears (depending on whether concomitant CIN is present or not, respectively), repeated at 6-month intervals. On each attendance at the Clinic, the patient is subjected to colposcopy accompanied by cervical swabs (for isolation of Chlamydia trachomatis), and by blood samples for viral (HPV, HSV, CMV) serology. PAP smears and punch biopsies are analysed for the cytopathic changes of HPV, and for concomitant CIN, and the biopsies also for HPV particles on EM, for HPV structural proteins with IP-PAP technique and for the local immune reactivity using ABC (Avidin-Biotin Peroxidase Complex) method with monoclonal antibodies (McAb) to define T cell subsets, NK (natural killer) cells and Langerhans ($OKT\text{-}6^+$) cells in the biopsies. All patients are requested for their sexual habits by a detailed questionnaire. Evidence was found by serological means (CF-test) for the coexistent HSV infection in 10% of the females during the follow-up. Chlamydial cervicitis was detectable in 10.4% of the patients, the figures being fully comparable with those reported by STD clinics. In HPV patients, the questionnaires disclosed the same risk factors (i.e. early onset of sexual activity, poor hygiene, and multiple partnerships) as shown to be associated with the development of cervical cancer, thus emphasizing the identical (STD) epidemiology of these two diseases. Only minor fluctuations in the relative levels of NK ($HNK\text{-}1^+$) cells and Langerhans ($OKT\text{-}6^+$) cells were observed between the different types of HPV lesions, between various grades of HPV-CIN, and between the HPV lesions with different natural history. The $OKT4^+/OKT8^+$ (T helper/T suppressor cell) ratio was lowest (0.93) in progressed lesions and highest (1.07) in the regressed ones, suggesting that the clinical course of cervical HPV infection might be affected by the local balance between T helper and T suppressor cells. During the follow-up, 86/343 (25.1%) of the HPV lesions regressed (RE), 209 (60.9%) remained persistent (PE), and 48 (14.0%) progressed (PR), 14 (4.1%) having been coned due to progression into CIS. The clinical course was most powerfully influenced by the grade of HPV-associated CIN, to which RE was inversely and PR directly related. The results unequivocally confirm the STD character of cervical HPV infection, which seems to possess the potential for clinical progression towards CIS, quite comparable to that of classical CIN. Therefore, cervical HPV lesions should be regarded as truly precancerous changes, and they should be examined, treated and followed-up with the same concern as the classical CIN.

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1310 HUMAN PAPILLOMAVIRUS DNA IN ANOGENITAL CARCINOMAS, Anna Marie Beckmann, Janet R. Daling and James K. McDougall, Fred Hutchinson Cancer Research Center, Seattle, WA 98104

Human papillomavirus (HPV) infection of the genital region is a common occurence in sexually active populations and epidemiologic evidence indicates that genital warts are a sexually transmitted disease. The epidemiology of cervical cancer suggests that this disease may also be transmitted by sexual contact and evidence from many laboratories has established an association between HPV infection and cervical malignancies. We are conducting case-control epidemiologic and biochemical studies of vulvar and anal cancers to investigate the relationship of HPV infection to the development of these malignancies.

We have used Southern transfer hybridization analyses to demonstrate evidence of HPV infection in patients with vulvar and anal cancers as well as in women with cervical cancer. DNA sequences closely related to HPV-16 or -18 were detected in 66% of vulvar cancers (4/6), 60% of anal cancers (3/5) and 56% of cervical cancers (5/9). The vulvar and anal tumors which contained HPV DNA were squamous carcinomas, and the positive cervical tumors were of either squamous or adenosquamous origin. In patients with vulvar or anal cancers that contained HPV DNA, development of malignancy was associated with preexisting or coexisting genital warts and with histories of anal intercourse. These studies allow incorporation of epidemiological and biochemical data into evaluation of the role of HPV in the induction of human genital malignancies.

1311 Characterization of the Involvement of HPV in Verrucous Carcinoma of the Larynx, Janet L. Brandsma, Allan L. Abramson, and Bettie M. Steinberg, Long Island Jewish-Hillside Medical Center, New Hyde Park, NY 11042.

We have previously shown by Southern blot hybridization that DNA sequences related to HPV-16 are associated with verrucous carcinoma of the larynx (6 out of 6 cases), while no detectable HPV-related sequences were found in 4 other squamous cell carcinomas of the larynx.

Experiments in progress aim at further defining the involvement of HPV in cancers of the head and neck. Tissues from two new cases of verrucous carcinoma and one cell line established from a third patient with this disease are being analyzed for the presence of HPV DNA. If found, we will molecularly clone the DNA(s) and characterize their homology to other types of cloned HPVs by analysis with restriction endonucleases and hybridization under varying conditions of stringency.

In addition, large numbers of tissues from other patients with malignant or benign diseases of the head and neck are being analyzed in our effort to estimate better the prevalence of HPV-11, HPV-16, HPV-18 and (if cloned) the verrucous type of HPV DNA.

1312 PROSPECTIVE HPV DNA ANALYSIS OF PATIENTS WITH ABNORMAL PAP SMEARS. Robert D. Burk, Anna S. Kadish, Servio Calderon, and Seymour Romney. Albert Einstein College of Medicine, Bronx, NY, 10461.

Human papilloma viruses (HPVs) have been implicated in an etiologic role for human cervix cancer. In order to investigate the relationship between HPVs, premalignant cervical lesions, and cervix cancer, we have used Southern blot analysis with HPV types 6, 11, 16, and 18 to detect HPV DNA in secretions and biopsy materials. All patients were referred to colposcopy clinic after at least two abnormal PAP smears. HPV DNA was detected in the cervical biopsy and/or fluid of 70% (16/23) of patients. 94% (15/16) of the HPV-positive patients showed koilocytosis and/or dysplasia on biopsy as well as abnormal PAP smears. Several patients had HPV in one cervical biopsy, whereas a second biopsy was negative both for HPV and for histopathologic changes. HPV 11 was the most common HPV type detected; at least 3 patients had more than one HPV type. 86% (6/7) of patients without detectable HPV DNA showed neither histologic evidence of HPV infection nor dysplasia; and 1 patient had dysplasia without koilocytosis. 35% (6/17) of HPV-positive patients had HPV DNA in cervical fluid samples but not in biopsy samples. All 6 of these patients had abnormal PAP smears on the day of fluid sampling. We conclude that (1) there is a close correlation between the presence or absence of HPV DNA in cervical biopsies and the histologic findings; (2) HPV infection is limited to discrete areas of the cervix with histologic abnormalities, whereas adjacent histologically normal areas are free of HPV DNA; and (3) analysis of cervicovaginal fluid is a sensitive, representative method for detection of HPV DNA infection of the cervical area. Comparison of histologic findings with HPV type will be presented.

1313 LOCAL CELLULAR IMMUNE RESPONSE AND KERATINOCYTE ANTIGEN MODIFICATIONS IN HUMAN PAPILLOMAS. Yvette Chardonnet, Jacqueline Viac and Paule Beauve. Inserm U 209, CNRS ERA 788, Hôpital E. Herriot, Pav. R, 69374 Lyon Cédex 08 France.

Ninety-two lesions taken from different localizations were studied by immunofluorescence (IF) with specific monoclonal antibodies (Ab), for the presence of T cell subsets, Langerhans cells (LC), HLA-DR bearing cells and keratincoyte antigens. The presence of papillomavirus (HPV) was examined either by IF with rabbit Ab raised to SDS-dissociated purifed virus (dHPV) and/or by viral DNA-DNA hybridization. The number of lesions containing T cell subsets differed according to their localization : plantar sole 2/15, hand 7/38, miscelleanous 6/16, genitals 4/7, laryngeal papillomas (LP) 2/8, bowenoid papules (BP) 6/8. T8 positive cells predominated in LP 2/8 and in BP 5/8 whereas both T cell subsets were equally found in the other biopsies. The distribution of LC was significantly disturbed as compared to normal tissues. The percentages of HLA-DR positive biopsies significantly differed. More than 60 % of biopsies contained viral antigen and/or viral DNA. As compared to normal epidermis, a number of papillomas showed some modifications of keratinocyte antigens : 55-57 Kd keratins, recognized by KL1 monoclonal Ab, were detected in basal cells ; membrane and desmosome associated antigens, reacting respectively with KL3 and KM48 monoclonal Ab, were drastically disturbed in the upper layers of epidermal lesions. Correlations were established between the presence of LC, dHPV and T8 cell subset. The various changes observed in papillomas are probably under control of viral infection. The modulation of T cell subsets might explain either regression or persistence of the lesions.

1314 MONOCLONAL ANTIBODIES TO THE GROUP SPECIFIC ANTIGEN OF PAPILLOMAVIRUS, Lex Cowsert and Phil Lake, Georgetown University, Washington, D.C., 20007.
Monoclonal antibodies were generated by immunizing C57Bl/6 mice with SDS-disrupted BPV-1. The primary screen was by ELISA designed to discriminate between antibodies that reconized determinants that were carried internally or externally on the virus capsid. This assay allowed the antibodies to be classified into three groups based on their reactivity. One group reacted only with intact virus, one group reacted only with disrupted virus, and the third group reacted with both intact and disrupted virus. Antibodies from the last two groups were screened by immunoperoxidase on human cutaneous warts for group specific activity. Initial surveys on formalin-fixed paraffin-embeded cervical biopies and Papanicolaou-stained cervicovaginal smears have demonstrated the ability of these antibodies to detect papillomavirus infection of the cervix. These monoclonal antibodies show greater sensitivity and specificity than the polyclonal antisera. Immunoprecipitation studies with these antibodies and charaterization of the antigen are being carried out.

1315 HISTOLOGICAL AND MOLECULAR ANALYSIS OF EARLY CERVICAL NEOPLASIA. Christopher P.Crum, Nobutaka Nagai, Masaru Mitao, Richard U. Levine and Saul J. Silverstein, Columbia University, New York, N.Y.
We have combined systematic histological analysis with Southern blot hybridization and DNA in-situ hybridization to correlate the presence of certain human papillomavirus (HPV) strains with specific precancerous cervical lesions and to pinpoint the distribution of HPV's in these lesions. In a series of 58 biopsies, HPV sequences were detected in 85% of the samples with abnormal histology, HPV 16 was detected in 70% of precancers which contained abnormal mitoses and/or diffuse nuclear atypia (CIN). Eighty two percent of condylomata contained HPV sequences, of these HPV 6 or 11 was detected in 56% of the lesions. Mixed infections were rare. DNA in-situ hybridization of biopsies from CIN and condylomata localized HPV sequences within nuclei, primarily in the superficial cell layers. However, in some lesions positive nuclei were found within one cell layer of the basal lamina and sequences hybridizing with HPV 16 were found in both mature and immature neoplastic cells. This study indicates that these viruses segregate within lesions which exhibit strikingly different histological changes. However, the lesions associated with these diverse viruses share similar patterns of distribution of HPV in the epithelial cells.

1316 DETECTION OF PAPILLOMAVIRUS IN TUMOURS USING LABELLED BIOPSY DNA. Ethel-Michele de Villiers, Referenzzentrum für humanpathogene Papillomviren, Deutsches Krebsforschungszentrum, Heidelberg.
A series of tumours were screened for the presence of papillomaviral DNA. After labelling 1ug of total biopsy DNA, hybridization with HPV 1 to 26 was carried out. Multiple infections by two or more viruses were further investigated by hybridizing the cellular DNA with the respective labelled papillomaviral DNA. New, apparently yet unidentified virus types crosshybridizing with known HPV prototypes were found in certain biopsies. The method permitted us to detect and identify specific types of papillomaviral DNA in tumours of the genital tract, the oral cavity and the skin, as well as a few additional lesions previously not suspected to result from papillomavirus infections.

1317 ORAL "HAIRY" LEUKOPLAKIA - EVIDENCE OF BOTH PAPILLOMAVIRUS & EPSTEIN-BARR VIRUS, J.S. Greenspan, D. Greenspan, E. Lennette, V. Petersen, & Y. de Souza, University of California, San Francisco, CA 94143 and Virolab, Emeryville, CA 94608
Sixty-one male homosexuals presenting with leukoplakia of the lateral margin and ventral surface of the tongue ("hairy leukoplakia" (HL) Greenspan, D et al., Lancet, II-1984) were subjected to immunological, histopathological and virological investigation. Thirteen patients developed AIDS during the 36 months of the study (at from 2 weeks to 33 months from first examination). 40/50 showed a reduced or absent delayed hypersensitivity skin test response while 19/20 had a Th/Ts ratio < 1. Biopsy of the lesion showed characteristic histology with koilocytosis. The PAP reaction for papillomavirus antigen was positive in 31/38 HL biopsies, in 4/7 oral warts and in 0/11 biopsies of other oral epithelial lesions. Electronmicroscopy revealed sporadic papillomavirus particles in 15/21 specimens. However, 17/21 specimens showed numerous herpes-type virus particles in nuclei, cytoplasm and intercellular spaces of prickle cells and more superficial keratinocytes. Efforts to culture the viruses have not been successful. Direct immunofluorescence on cryostat sections, using rabbit antisera to herpes simplex (HSV) 1 or 2, or varicella-zoster virus (VZV) and indirect immunofluorescence using human sera containing antibodies to cytomegalovirus (CMV), showed no positive staining in any case. Human sera positive for Epstein-Barr virus (EBV), used in indirect immunofluorescence, consistently produced staining in koilocytotic and adjoining cells in 13/15 HL cases and in 0/11 specimens of other oral epithelial lesions. HL is a new entity and represents oral leukoplakia of probable viral origin in immunosuppressed male homosexuals. The lesion appears to contain both human papillomavirus and Epstein-Barr virus and may be predictive of AIDS. Supported by grants from the UC Systemwide Taskforce on AIDS.

1318 TRANSMISSION OF GENITAL PAPILLOMAVIRUS INFECTIONS: A STUDY OF SEXUAL PARTNERS, Gerd Gross[1], Achim Schneider[2], Barbara Hauser-Brauner[1,3], Dieter Wagner[1], Hans Ikenberg[1], Lutz Gissmann[3], [1]Universities of Freiburg and [2]Ulm, [3]Deutsches Krebsforschungszentrum Heidelberg, FRG.

Heterosexual Partners of 18 men and 31 women with either condylomatous lesions or intraepithelial neoplasia of the penis (PIN; Bowenoid Papulosis, BP), of the cervix (CIN) and of the vulva (VIN, BP) were examined macroscopically, histologically, cytologically and virologically using a recently described in situ hybridization method for detection of typespecific HPV-DNA sequences in cells from penile, cervical and vulvar smears (Wagner, D., pers.comm.).
In contrast to 44 % (8/18) of female partners of men with the mentioned lesions only 29 % (9/31) of male partners of females presented clinically evidence for a papillomavirus infection. Overall in 51 % (25/49) HPV-DNA was detected in cells of at least one of the two partners, HPV 16 was present in 14,3 % (7/49) and HPV 11 in 6,1 % (3/49) of both partners, respectively.
Concluding, when men were seen first with HPV-associated lesions their female partners had a higher rate of HPV-positive genital lesions than viceversa. As a possible reason for this discrepancy the occurence of clinically occult lesions in men is discussed according to one case with intraurethral HPV-16 positive papillomas. Another explanation might be a higher regression rate of such lesions in male patients.

1319 UTILIZATION OF FLOW CYTOMETRY TO QUANTITATE DNA AND T ANTIGEN IN SV_{40} INFECTED AND TRANSFORMED CELLS, John M. Lehman, David Fogleman and James W. Jacobberger, Department of Pathology, University of Colorado School of Medicine, Denver, CO 80262

The papovavirus, SV_{40}, is capable of lytically infecting and transforming rodent and primate cells. Recently, research interest has focused on the activity of the viral T antigen(s) and their relationship to the viral replication cycle or the transformed state. Flow cytometry offers a means to quantitate several biological phenomena simultaneously on a single cell basis. We have used propidium diiodide to quantify DNA and an immunofluorescent assay using antibody tagged with fluorescein isothiocyanate to quantify T antigen. Chinese hamster cells transformed by SV_{40} were utilized in these studies. Cells were fixed and stained with anti-T serum or monoclonal antibody followed by anti-IgG-FITC. Microscaling the technique permits the processing of as few as 10,000 cells, but we routinely use 500,000. Assay improvement and computer analysis were utilized to minimize the background fluorescence resulting in a T antigen-positive population with a signal ≈70% over background. The data to be presented will compare the amount of T antigen present in the cell with the state of the cell cycle and growth rate of the population. These studies have been performed on cloned wild-type and tsA-mutant transformed cells. The development of this technique for quantitation of this specific viral antigen correlated with the stage of the cell cycle allows the characterization of the relationship that T antigen has to cell growth and proliferation. (This work was supported by grants CA-16030, CA-15823, CA-09157 and a departmental gift from R.J. Reynolds Industries, Inc.)

1320 PAPILLOMAVIRUS GENOMES IN HUMAN CERVICAL CARCINOMAS: ANALYSIS OF THEIR TRANSCRIPTIONAL ACTIVITY, Hermann Lehn, Peter Krieg and Gerhard Sauer, Institute for Virus Research, German Cancer Research Center, 6900 Heidelberg, FRG

The DNA of individual types of human papillomaviruses (HPV) is found in some malignant human tumors, e. g. laryngeal carcinomas, epidermodysplasia verruciformis and cervical cancer. In the present study, six human cervical carcinomas were analyzed for the presence of HPV DNA and RNA. Four of the tumor specimens were shown to harbor the DNA of HPV16 as a single copy per cell exclusively in an integrated state. The two remaining tumors as well as normal uterine and cervical tissue from two of the patients with HPV16-containing tumors lacked detectable HPV16 DNA. There is compelling evidence for the monoclonal origin of three cervical carcinomas that were analyzed. Search for HPV16 RNA species revealed the presence of HPV16 mRNA in only one out of the four tumors, while we failed consistently to detect HPV16 specific RNA sequences in the remaining three specimens under condition which permitted the detection of less than one mRNA molecule per cell. The possible role of HPV16 in the development of human cervical cancer will be discussed.

1321 HPV16 genomes in males and their female partners Dennis J. McCance, Michael Campion, Albert Singer, Richard Doll

Epidemiological evidence indicates that premalignant and malignant disease of the cervix is sexually transmitted. We will present data on the frequency of HPV16 in premalignant and malignant disease in the U.K. and the prevalence in the male consorts of some of these women. Further information on HPV16 and 18 infection of males from a province of Brazil where the incidence of carcinoma of the penis is as high as that of the cervix will also be presented. Here the prevalence of HPV16 and 18 in biopsies from penile carcinomas is 44% (21/48) and 24% (6/25) respectively. The incidence of HPV types in females from the same population is being assessed.

1322 PRESENCE OF HUMAN AND HPV DNA SEQUENCES IN MOUSE EPITHELIAL CELLS TRANSFECTED WITH HUMAN CONDYLOMA DNA, Don M. Morgan, Gene Pecoraro and Vittorio Defendi, NYU Medical Center, New York, NY 10016

Several distinct types of human papillomaviruses have been correlated with benign and malignant lesions of the genital tract. To study the oncogenic functions of papillomaviruses associated with these lesions, we have transfected various species of primary and established cell lines with either cloned HPV DNA molecules or high molecular weight ano-genital tumor DNA containing HPV sequences.

So far, none of the cell lines transfected with cloned HPV6 or 11 molecules were susceptible to transformation when assayed by growth in foci or colony formation in agar. We purified high molecular weight DNA from ano-genital condylomas progressing to squamous cell carcinoma which contained HPV-6a episomal molecules at high copy number as well as additional heterologous HPV molecules which weakly hybridized to HPV-6 DNA probes. Cell lines were transfected with the tumor DNA in the hope that the human genomic DNA would supply sequences which would facilitate transformation by the HPV DNA. After transfection of Pam 212 mouse epithelial cells, we obtained colonies in agar at increased frequency over controls and amplified these into cell lines, at least one of which contains HPV and human DNA sequences. We are currently analyzing the HPV and human DNA present in these cells as well as their ability versus control cells to induce tumors in syngeneic mice. (Supported by NIH grants #CA 16239 and #CA 09161).

1323 MONOCLONAL ANTIBODIES TO PAPILLOMAVIRUS CAPSID PROTEIN. Yutaka Nakai, Wayne D. Lancaster and A. Bennett Jenson. Georgetown University, Washington, D.C. 20007

Myeloma hybridomas were obtained by fusing the mouse myeloma P3X63Ag8U.1 with spleen cells from BALB/c mice immunized with sodium dodecyl sulfate (SDS)-disrupted virions of bovine papillomavirus type 1 (BPV-1). Nine hybridoma cell lines were established after screening by the enzyme-linked immunosorbent assay (ELISA) using SDS-disrupted BPV-1 as the antigen and indirect immunofluorescence (IIF) tests on frozen sections of BPV-1 induced bovine fibropapillomas. Supernatant from one of these cell lines was reactive with papillomavirus common antigens as detected by positive IIF on frozen sections of BPV-1, BPV-2 fibropapillomas and human plantar warts and by avidin-biotin complex tests on formalin-fixed anogenital condylomas of mild cervical dysplasias. Seven monoclonal antibodies were reactive by IIF with both BPV-1 and BPV-2 fibropapillomas, and one was reactive only with BPV-1 fibropapillomas. SDS-polyacrylamide gel electrophoresis followed by Western Blots showed that all monoclonal antibodies reacted with the major capsid protein (MW 53,000) of BPV-1.

1324 EQUINE PAPILLOMAVIRUS: PARTIAL CHARACTERIZATION AND PRESENCE IN COMMON EQUINE SKIN TUMORS: M. Kerry O'Banion, John P. Sundberg, and M. E. Reichmann, University of Illinois, Urbana, IL 61801

Equine papilloma virus was isolated from cutaneous papillomas of several ponies. Virus particles were observed by transmission electron microscopy of thin sectioned tissue and structural viral antigens were detected immunologically using the peroxidase-antiperoxidase technique. Purified virions isolated from tissue extracts exhibited characteristic papilloma virus morphology (55 nm icosohedrons) in negative stained preparations. The structural proteins were characterized by SDS-PAGE under reducing conditions and found to be similar in their mobilities to those of BPV-1 and BPV-2. Based on melting temperature determination, DNAs from these three viruses were judged to have similar GC contents.

Purified viral DNA was cloned by its single Bam Hl site into pBR322. A detailed restriction map of the viral DNA was determined. Using non-stringent annealing conditions with specific BPV-1 restriction fragments as probes, the genomic organization of EqPV was derived. Liquid hybridization with BPV-2 probes under stringent conditions (42°C, 50% formamide, 0.825 M Na^+) indicated a 15% homology between the two viral DNAs. The nature and possible relevance of this homology to the reported presence of BPV in equine sarcoids will be discussed.

1325

HUMAN PAPILLOMA VIRUS DNA SEQUENCES IN CERVICAL CARCINOMA CELL LINES

Mary M. Pater and Alan Pater. Molecular Biology/Microbiology, Health Sciences Center, Memorial University of Newfoundland, St. John's Newfoundland, Canada

A total of eight permenant human epithilial cell lines established from cervical carcinoma were analyzed by dot-blot hybridization for the presence of human papillomaviruses (HPV), types 16 and 18 (Kindly provided by Gissman and ZurHauzen). HPV DNA sequences were detected in six cell lines. Using Southern blot analysis and either HPV 16 or 18 DNAs as probes, we detect HPV 18 in four of the positive cell lines and HPV 16 in two other cell lines. None of the cell lines containing HPV 18 have the complete genome of this virus. All the cell lines contain the early region E1, E6 and E7, but are missing the early region E2, E4 and E6. One cell line containing HPV 16 has the complete genome of the virus in both free and integrated form. The other cell line contains most of the HPV 16 sequences in an integrated form. Further analysis of the state viral DNA in these cell lines is under investigation.

1326 ENHANCED ANTIGEN FOUND IN BPV-DNA INFECTED MOUSE CELL CULTURE LINE, S. Kalinowski and D.E. Pumo, Biology Department, Hofstra University, Hempstead, NY 11550
A hybridoma was obtained which secreted a low level of antibody capable of differentiating between BPV-DNA infected ID13 and non-infected C127 mouse cell polypeptides by activity in enzyme-linked immunosorbent assay (ELISA). The ELISAs demonstrate that some antigen is present in the C127 chromatin, however more is found in the ID13 cell chromatin. Other sample cells tested including 3T6 fibroblasts, and non-culture adapted rat liver also contain some antigen, however the amount is lower than in the ID13 cells. Human condyloma chromatin was tested in ELISA; at least three times as much antibody was bound per ug chromatin than was bound in ID13 cell chromatin. The antibody was tested by immunoblots to determine the molecular weight of the antigen polypeptides. The antibody binds to three polypeptides present in both ID13 and C127 cells; however, the antibody binding to the 30K MW polypeptide is considerably enhanced in the ID13 cells. We also performed immunocytochemistry to localize the antigen. Four distinctly different staining patterns were obtained: 1) staining in the perinuclear area, no nuclear staining, 2) similar to (1) except with dark grains in the perinuclear area, 3) heavy staining in perinuclear area (cells not completely flattened), 4) little cytoplasmic staining, prominent reticular staining in the nucleus. We have been unable to determine the factors controlling localization. Parameters tested include cell cycle association and the possibility that the antibody is recognizing a "heat shock" or stress protein. (This work was supported by PHS grant no. 1PO1NS19214-01, NSF grant no. RII-8217798, NYHRC grant no. C-000272.)

1327 IDENTIFICATION OF GENITAL TRACT PAPILLOMAVIRUS GENOMES IN LESIONS OF THE ORAL CAVITY, Z. Naghashfar, E. Sawada, M.J. Kutcher, J.R. Swancar, J. Gupta, R. Daniel, H. Kashima, J.D. Woodruff and K.V. Shah, Johns Hopkins Medical Institutions, Maryland State Cancer Control Unit and the University of Maryland School of Dentistry, Baltimore, MD

Warts in the oral cavity are often diagnosed as verruca vulgaris, condyloma acuminatum, solitary wart, epithelial focal hyperplasia, and other conditions on the basis of their clinical and histologic characteristics. Because the oral cavity and the genital tract share many sexually transmitted pathogens, we are screening oral cavity lesions using radioactive DNA probes of genital tract viruses HPV-6, HPV-11, HPV-16 and HPV-18. In tests of extracted tissue DNAs with ^{32}P-labelled HPV-6 probe in non-stringent filter hybridization tests, papillomavirus-related DNA sequences were demonstrated in 3 of 7 oral tissues examined. The genome in one of the positive tissues was identified as HPV-6, subtype a, by its strong reactivity with an HPV-6 probe in stringent hybridization tests and by the restriction endonuclease digest patterns after incubation with PstI and other enzymes. The warts which yielded HPV-6a were diagnosed histologically as verruca vulgaris, and they occurred as multiple, asymptomatic papillary lesions of many years duration in a 14 year old black male. HPV-6, subtype undetermined, was recovered from a single papilloma on the posterior surface of the tongue of a young white female. The genotype of the third positive lesion, also from a young white female, is undetermined but it appears to form stable hybrids with a pool of HPV-16 and HPV-18 probes. We are now screening paraffin sections of previously collected pathological tissues from the oral cavity by in situ hybridization with 35S-labelled probes to identify papillomavirus genotypes which infect the oral cavity. (Supported by NIH grant PO1 AI-16549.)

1328 IMMUNOLOGICAL ASPECTS OF BOVINE INFECTION WITH BPV3. Peter B. Spradbrow and Vijay K. Kuchroo, University of Queensland, Brisbane, Australia.

Calves were infected with BPV 3 and tested for the development of cell mediated immunity and of serum factors that block cell mediated immunity. Comparisons were made with bovine ocular squamous cell carcinomas - malignancies which develop from papillomas and in which papillomavirus genomes have been detected. The erythrocyte-rosette augmentation (ERA) assay was used to measure the responsiveness of leukocytes to antigens.

Leukocytes from cattle with squamous cell carcinomas reacted only with squamous cell carcinoma extracts and leukocytes from cattle with cutaneous papillomas reacted only with papilloma extracts. Autologous serum from cattle with carcinomas blocked antigen-specific ERA and autologous serum from cattle with papillomas blocked antigen-specific ERA. Serum from carcinoma-bearing cattle blocked carcinoma-specific ERA in some allogeneic combinations but it did not block papilloma-specific ERA.

1329 FATE OF VIRAL DNA IN SV40 INFECTED HUMAN EPIDERMAL KERATINOCYTES, Mark L. Steinberg, NYU School of Medicine, New York, NY 10016

We analyzed the state of the genomic DNA of the papovavirus, SV40, in human keratinocytes as viral infected cells gradually acquired a transformed phenotype over time. Initially, the vast majority of the viral DNA is maintained either in a full length supercoiled form or as truncated subgenomic fragments with little evidence of integration. However, analyses of clonal populations revealed great heterogeneity and instability of the viral DNA and we were able to isolate one clonal subpopulation in which integrated forms of the virus appeared to predominate. Similarly, uncloned populations eventually ceased production of the "free" viral DNA after several years in culture and instead came to display tandemly repeated SV40 copies at a single host integration site. Interestingly, Bgl II digestion of host DNA generated restriction fragments containing the integrated SV40 DNA which were of differing sizes in cultures at the 144th vs. the 163rd serial passage suggesting modification or rearrangement of sequences at or near the integration site. Host sequences flanking the integrated viral DNA at the 163rd serial passage have been isolated on restriction fragments of < 5Kb generated by either Bam HI or Hpa II digestion. (Supported by NIH grants #CA 27869 and CA 32485)

1330 EXPRESSION OF HUMAN TISSUE PLASMINOGEN ACTIVATOR IN MOUSE CELLS, Paul E. Stephens, Mary M. Bendig, Christopher C. Hentschel, Celltech Limited, Slough SL1 4DY, U.K.

We have evaluated the usefulness of different promoters and terminators for expressing the human tissue plasminogen activator (tPA) gene in mouse C127 cells. The tPA cDNA gene was linked by transcriptional fusion to promoters such as the mouse metallothionein (MMT) promoter, the Rous sarcoma long terminal repeat (LTR), or the mouse Moloney leukaemia virus LTR. Terminators were provided by linking the 3' end to DNA fragments containing the MMT polyA site or the SV40 early polyA site. The reconstructed tPA genes were inserted into bovine papilloma virus (BPV) vectors containing the BPV genome, the MMT gene, and bacterial plasmid DNA. Cells were transfected with the DNA constructs and tPA-producing foci were easily identified using a novel application of the fibrin-agarose assay method for detecting tPA activity. Stably-transformed, tPA-producing cell lines were analyzed in detail for the physical state and copy number of the tPA gene in the cells, the level and authenticity of tPA transcription, and the amount of active tPA protein being secreted.

1331 SQUAMOUS CELL CARCINOMA ASSOCIATED WITH CANINE ORAL PAPILLOMAVIRUS, John P. Sundberg, Elizabeth Schmidt, Kerry O'Banion, University of Illinois, Urbana, IL 61801, and Carl Olson, University of Wisconsin, Madison, WI 53706

Use of a live canine oral papillomavirus (COP) vaccine in a commerical kennel resulted in development of cutaneous squamous cell carcinomas at the site of I.M. inoculation. Latency ranged from 2 to 3 years and frequency was low. Five of 12 tumors were positive for the presence of papillomavirus group specific antigens by PAP. One hypothesis is that epithelium in which the virus can replicate is pushed into the muscle during injection. Some cells are infected, survive and ultimately transform, resulting in a slow-growing, locally-invasive squamous cell carcinoma. Subcutaneous papillomatous cysts have been produced in this way in cattle with bovine papillomavirus (Koller and Olson, 1971). To test this, five susceptible male Beagles from another source were inoculated in several locations with a crude homogenate of the original oral papilloma used to make the vaccine. Four dogs developed oral papillomas and one developed an ocular papilloma, but after one year no carcinomas have been observed at the site of I.M. injection of minced autologous haired skin or oral mucosa mixed with the viral suspension. Restriction digests of COPV DNA purified from the original papilloma yielded a pattern similar to that originally published by Pfister and Meszaros (1980). The viral DNA has been cloned and a detailed restriction map prepared. This clone is being used to probe, by Southern Blot hybridization, DNA from induced tumors in this colony as well as spontaneous canine squamous cell carcinomas and papillomas.

1332 DETECTION AND CHARACTERIZATION OF A NEW TYPE OF HUMAN PAPILLOMA VIRUS, Attilla T. Lorincz, Wayne D. Lancaster and Gary F. Temple, Bethesda Research Laboratories, 8717 Grovemont Circle, Gaithersburg, MD 20877

We have identified and molecularly cloned a new type of papilloma virus, HPV31,which is commonly associated with anogenital neoplasms. HPV 31 lacks high sequence homology to other known types of HPV; it does contain limited regions of partial homology which are revealed by hybridization at Tm-35°C. Detailed restriction mapping and an analysis of the genomic organization of HPV31 are underway.

In our study of specimens obtained from the Washington DC area, HPV31 was present in approximately 35% of mild cervical dysplasias but in less than 5% of CIS and cervical carcinomas whereas HPV types 6 and 11 were detected only rarely in cervical dysplasias. In contrast, studies by others in Germany and Britain detected a much higher proportion of HPV types 6 and 11 in mild and moderate dysplasias. We conclude that in the population of women that we studied, HPV31 is one of the most common types of papilloma virus infecting early dysplasias of the cervix.

1333 THE PRESENCE OF HPV SEQUENCES IN THE UTERINE CERVICES OF WOMEN WHOSE CERVICAL SMEARS ARE NORMAL OR SHOW INFLAMMATORY CHANGES. Philip G. Toon, John R. Arrand and David S. Sharp, Paterson Laboratories, Manchester M20 9BX,UK.

It has been observed that some patients with recent previously normal cervical smears or smears showing inflammatory changes only, have rapidly progressed to a major degree of C.I.N. or overt cervical carcinoma. It is known that HPV sequences are present in a large proportion of C.I.N. or cervical carcinomata. Consequently, it is now widely believed that HPV has a role in the aetiology of these lesions, possibly in conjunction with some promoting factor.

We have initiated an investigation into the sexual behaviour of these patients and the prevalence of HPV sequences in their cervical tissue. Other agents such as bacteria which might cause inflammatory changes are also being investigated.

The aim is to ascertain if these cervical changes are due to HPV or other agents and to compare these with a series of normal cervices. If there is an increased incidence of HPV in these inflammatory smears this would support the hypothesis that this is an at risk group which should be followed up closely. The current status of the ongoing study will be presented.

1334 FIBROBLAST IMMORTALITY IS NOT A PREREQUISITE FOR TRANSFORMATION BY BOVINE PAPILLOMAVIRUS TYPE-2, Barbara A. Watt, Helen M. Laird, William F.H. Jarrett, University of Glasgow.

Non-passaged primary cell cultures of foetal bovine skin and palate fibroblasts were infected with BPV Type 2. Morphological alterations were observed in the skin and palate cells after 11.6 and 7.4 population doublings respectively. The cells appeared long and spindle shaped and exhibited properties characteristic of cellular transformation including loss of contact inhibition, anchorage independant growth and tumorigenicity in nude mice. Non-integrated BPV-2 DNA sequences were also detected in the transformed cells.

It has therefore been demonstrated that BPV Type 2 possesses the capability of fully transforming bovine fibroblasts that have not become adapted for short term grŏwth in vitro nor developed the potential for indefinite subculture.

1335 PRESENCE AND EXPRESSION OF HPV DNA SEQUENCES IN HUMAN CERVICAL CARCINOMA LINES, Indira Krishnan-Hewlett, Carole Yee, Richard Schlegel, and Peter M. Howley, Laboratory of Tumor Virus Biology, NCI, Bethesda, Maryland 20205

A series of human carcinoma cell lines was examined for HPV DNA sequences using HPV-6, HPV-11, HPV-16, and HPV-18 DNA probes. Each of the eight cell lines derived from human cervical carcinomas contained integrated HPV DNA sequences. In six of the eight lines, HPV-specific polyadenylated RNA species were identified. The expression of HPV sequences was detected in four lines with a HPV-18 DNA probe and in two lines with a HPV-16 DNA probe. The two cell lines containing HPV-16 specific transcripts contained integrated viral sequences which hybridized to both HPV-16 and HPV-18 DNA probes. The viral transcripts expressed in these cell lines are currently being mapped. Four cell lines established from human squamous cell carcinomas of the bladder, pharynx, lung, and vulva were negative for HPV-6, HPV-11, HPV-16, and HPV-18 DNA sequences.

Viral Vectors

1337 USE OF BOVINE PAPILLOMA DERIVED VECTORS TO STUDY REGULATION OF GENE EXPRESSION, Michael Karin, Adriana Heguy, Simon Ford and Alois Haslinger Department of Microbiology, USC School of Medicine 2011 Zonal Avenue, Los Angeles, CA 90033

The use of reversed genetics has contributed a great deal to our understanding of gene expression in higher eucaryotes which are not amenable to conventional genetic analysis. The almost standard approach is to introduce by transfection the gene of interest and its in vitro constructed mutants into cultured cells and then examine their expression. However, certain properties of the two most widely used systems: transient expression and stably transformed cell lines, complicate such an analysis. During transient expression only a fraction of the molecules introduced into the cells are transcriptionally active and in stably transformed lines the DNA sequences which flank the integration sites of transfected genes can exert strong modulatory effects upon their expression.

The use of BPV derived vectors allows the investigator to circumvent some of these problems, mostly because such vectors can persist as relatively low copy number episomes in transformed cells.

We are using BPV derived vectors to study the regulation of the human metallothionein (MT) genes. These genes belong to a multigene family encoding low molecular weight heavy metal binding proteins. The transcription of these genes is induced by group IB and IIB heavy metal ions, glucocorticoid hormones and various lymphokines such as interleukin 1 and interferons. To characterize cloned human MT genes as being functional, we insert them into a BPV vector and test their ability to protect transformed cells against Cd toxicity. Such vectors allow the cloned genes to be expressed in very high levels and if these genes encode a functional heavy metal binding protein, their expression confers a heavy metal resistant phenotype upon the host.

Comparison of individual rat fibroblast cell lines carrying the human MT-II_A gene either stably integrated into the genome or carried on a BPV derived episome reveals extreme heterogeneity of expression phenotypes for stably transformed cell lines while BPV transformed cells exhibit a much more consistent phenotype in respect to MT gene expression.

In all cell lines containing MTII-BPV vectors, expression of the human gene is induced by cadmium ions, about 5-10 fold above its already high basal level. We are currently using such cell lines to examine the effect of induction on the topology of the episomes by resolving topoisomers on chloroquine gels. Also these episomes are used in methylation protection experiments to examine the interaction of regulatory proteins with the h MT-II_A gene before and after its induction.

1338 TRANSFORMING FUNCTIONS OF EPSTEIN-BARR VIRUS, Bill Sugden, John Yates, and David Reisman, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, WI 53706

Epstein-Barr virus (EBV) infects human B-lymphocytes in vitro and efficiently induces and maintains cell proliferation in the infected cell. The viral DNA is usually detected in the proliferating or "transformed" cells as complete plasmids which are maintained through serial cultivation of these transformed cells. We wish to identify and characterize viral functions that are required by EBV to transform B-lymphocytes. We have taken one path towards this goal by first identifying viral genetic elements that are required to maintain DNA as plasmids in mammalian cells. There are two such viral elements: one acts in cis and is required for replication of plasmid DNAs; the second encodes the nuclear antigen, EBNA-1, which interacts with the cis-acting element to permit replication of plasmid DNAs. We term the cis-acting element oriP for origin of plasmid replication. We have not shown that DNA synthesis begins within oriP, only that oriP is required in cis for DNA replication. oriP itself is composed of two cis-acting elements; one contains 20 tandem copies of a 30 bp sequence; the other, about 1 kbp away, contains a 65 bp inverted repeat with symmetrically positioned, partial copies of the 30 bp sequence. Derivatives of oriP have been generated in vitro which when tested in vivo indicate that the distance between its two elements can be eliminated or increased to 2.5 kbp without loss of function. Neither element serves as an enhancer of transcription. The gene for the nuclear antigen, EBNA-1, maps 100 kbp away from oriP on the viral genome. When this gene is linked to oriP and to a marker that is selectable in eukaryotic cells, such a recombinant plasmid can replicate stably in established dog, monkey, and human cell lines that neither contain EBV nor are infectable by this virus. These plasmids are usually maintained at the level of 1-20 copies per cell in the presence of the selective agent. These results indicate that we have identified the genetic elements of EBV that are necessary to maintain DNA as plasmids in a variety of cells. We are now adding to this mini-replicon other viral DNA in order to identify the minimal viral information required to transform human B-lymphocytes.

Plasmids derived from PBR322 that express resistance to hygromycin B or G418 in mammalian cells and contain oriP and the EBNA-1 gene can be used as shuttle vectors since they are maintained as plasmids in both prokaryotic and eukaryotic cells. Such vectors have a wider host range than do those derived from BPV but do not replicate in rodent cells. They should be useful vehicles for introducing and retrieving genes from a variety of mammalian cells.

DNA Replication and Genome Organization

1339 PRESENCE AND EXPRESSION OF BPV-4 IN TUMOURS OF THE ALIMENTARY CANAL OF CATTLE AND ITS POSSIBLE ROLE IN TRANSFORMATION AND MALIGNANT PROGRESSION.

M.S. Campo[1], W.F.H. Jarrett[2], M.H. Moar[3], and K.T. Smith[1].
1. The Beatson Institute for Cancer Research, Garscube Estate, Glasgow
2. Department of Veterinary Pathology, Veterinary School, Garscube Estate, Glasgow
3. Department of Zoology, University of Edinburgh, Edinburgh

BPV-4 induces papillomas of the upper alimentary canal, which transform to carcinomas with a very high frequency in cattle grazing on bracken fern. The progression to malignancy has been ascribed to the interplay between the virus and co-carcinogens present in the fern. To elucidate the role of the virus in the transformation process we have analysed several different malignancies of the alimentary canal and metastatic deposits for the presence of viral DNA. We have found the viral genome in only one case of tongue carcinoma, where it was present in a few episomal copies. All the other cases were negative even in hybridization conditions where detection of 0.01 genome equivalent per cell was possible. We conclude that, although the virus is the causetive agent of papillomas and therefore responsible for one of the primary events of cellular transformation, the presence of its genome is not required for the maintenance of the transformed state.

To understand how BPV-4 induces heritable cellular changes, we have analysed the transcription of its DNA in the pre-malignant papillomas, and have identified seven viral mRNA, ranging in size from 4.2 kilobases (kb) to 1.0 kb. By nucleotide sequence analysis and aminoacid sequence comparison (Patel, Smith and Campo, these abstracts) we have identified the RNAs coding for the late (structural) proteins and those coding for the early (transforming) ones. One of the transforming RNAs, the 1.0 kb transcripts corresponding to the E2 open reading frame, is present in very high amounts in papillomas, approx. 0.5% of the total polyadenylated RNA, but is absent in in vitro transformed mouse fibroblasts. This raises the interesting possibility that expression of E2 is specific for epithelial cells, and that the E2 protein is involved in keratynocyte transformation. Experiments aimed at the understanding of the role of this viral protein are in progress.

1340 SEARCH FOR THE BIOLOGICAL FUNCTION OF THE EARLY PROTEINS OF PAPILLOMA VIRUSES, Olivier Danos, Isabelle Giri, Francoise Theirry and Moshe Yaniv, Department of Molecular Biology, Pasteur Institute 75724 Paris Cedex 15, France.

The complete nucleotide sequence of several papilloma virus genomes was established in recent years. These viruses share a common organization of the early and late regions of their genome; six to seven open reading frames are found in the early coding region. However until now, no early viral protein was detected in infected or transformed cells. We undertook the analysis of the coding potential of the early region by examining the viral RNA present in cottontail rabbit papilloma virus (CRPV) induced carcinoams. We could show that the major RNA species present in VX2 cells are polycistronic; they can code in their 5'proximal portion for E6, truncated E6 and E7 open reading frames. If the second open reading frame in such a polycistronic message can be translated, it will yield a fusion protein between the N-terminal 3 aminoacids of E1 and the E4 open reading frame. Only minor mRNA species that we observed cover the entire E2 and E4 region (1).

Comparison of the aminoacid sequence of the open reading frames of papilloma viruses with known protein sequences showed homology between the C-terminal part of the E2 region and cellular or viral mos oncogene. The CRPV E6 open reading frame shows an intriguing homology with the beta-subunit of ATP synthase from mitochondria or chloroplasts (2) further studies to identify the viral early proteins and the control mechanisms of their synthesis in keratinocytes are in progress.

1 - O. Danos, E. Georges, G. Orth and M. Yaniv. submitted for publication.

2 - I. Giri, O. Danos and M. Yaniv. Proc. Natl. Acad. Sci. USA (in press).

Papilloma Viruses and Human Cutaneous Diseases

1341 PRESENCE AND POSSIBLE INVOLVEMENT OF HPV DNA IN PREMALIGNANT AND MALIGNANT TUMORS. Anthony J. Faras,[1] Ronald S. Ostrow,[1] Karen Zachow,[1] Takashi Okagaki,[2], Michio Fukushima,[2] Leo Twiggs,[2] and Michito Niimura.[3] Departments of Microbiology,[1] Obstetrics and Gynecology,[2] Laboratory Medicine and Pathology,[2] University of Minnesota Medical School, Minneapolis, MN and Department of Dermatology,[3] Jekei University School of Medicine, Tokyo, Japan.

We have recently completed a survey of well over 400 neoplasias from a wide variety of anatomical sites for the presence of HPV-related nucleotide sequences, employing Southern blot and in situ hybridization procedures. Whereas the vast majority of the benign and malignant tumors from the alimentary tract, lungs, ovaries, bladder, liver and other internal organs lacked detectable HPV-related genetic sequences, two groups of neoplasias consistently exhibited the presence of HPV-related nucleotide sequences: 1) squamous cell carcinomas from patients with the chronic flat wart disease syndrome epidermodysplasia verruciformis (EV), and 2) premalignant and malignant tumors of the anogenital tract. In the former group of neoplasias, all of the primary and metastatic carcinomas tested contain HPV DNA, indicating the association of HPV DNA with 100% of the tumors. In the latter neoplasias, the presence of HPV-related sequences has been established in a variety of premalignant and malignant tumors, although in some instances less reproducibly than in EV-squamous cell carcinomas. Of the 250 cases of cervical, vaginal and vulvar premalignant and invasive lesions analyzed for the presence of HPV DNA, the presence of these sequences could be identified in mild, moderate and severe dysplasia of the uterine cervix, Bowen's disease and Bowenoid atypia, condylomatous carcinoma of the vulva, vaginal dysplasia and carcinoma in situ, invasive carcinoma of the uterine cervix and frank invasive squamous cell carcinoma of the vulva. Although the presence of HPV DNA can be consistently found associated with certain premalignant and malignant tumors of the skin and anogenital tract, the involvement of HPV in the development of the malignant tumor is presently indeterminant. DNA transfection studies using molecularly cloned HPV-5 DNA obtained from a carcinoma from a patient with EV indicated that HPV DNA exhibited oncogenic potential and therefore exhibited the capacity to facilitate malignant progression. However, neither changes in the structure of the HPV genome nor its expression can be related to malignant progression with papillomaviruses to date.

Epidemiological studies have indicated high risk of HPV infection among the sexually promiscuous and infected partners, suggesting that sexual contact may reflect one mode of transmission. We have obtained data supporting this contention by demonstrating the presence of HPV DNA in semen from patients in high risk families or exhibiting debilitating chronic wart disease.

S. Jablonska, C. Croissant, G. Orth

1342 The clinical morphology, pathology and immunology of papillomavirus infections of the skin, as related to the virus type

The clinical morphology of HPV induced skin lesions depends on virus type. There is a remarkable plurality of HPVs demonstrated by molecular cloning /at present 34 HPV types/, and a preferential association with specific HPV types has been firmly established. New morphologic varieties of warts were found to be induced by HPV4 and HPV7, intermediate warts sharing features of common and plane warts are associated with HPV10 and HPV28. A variety of unusual HPV-induced lesions may be found in immunosuppressed persons. The natural history of HPV cutaneous infections and the immune response of the host differ considerably depending on the virus type. Clinical recognition is not always possible. However, there is almost a perfect correlation between histologic features and HPV type, if a proper lesion is chosen for biopsy. A specific histological marker of each HPV type is the cytopathic effect due to viral DNA replication, as demonstrated by molecular hybridization in situ, and to its interaction with the keratinocyte differentiation. Only the related HPVs 3, 10 and 28, sharing the same cytopathic effect, are not infrequently indistinguish able. Contrarily to the HPV infections in general population, in epidermodysplasia verruciformis /EV/, associated with EV-specific viruses, diverse cutaneous lesions have similar histologic features, with analogous cytopathic effect. However EV cases due to HPV3 and HPV10, i.e. viruses inducing also plane warts in general population, differ in clinical morphology and cytopathic effect. Malignant transformation in EV was found to be mainly associated with HPV type. Depression of cell mediated immunity /CMI/ was similar in cases induced by diverse EV-specific HPVs with known /HPV5, 8, 14/ or not proven oncogenic potential, and in cases induced by HPV3. The load with viruses is a further factor decreasing CMI, as shown in a case of regressing HPV3-induced EV. Increased natural killer cell activity appears to be an important host defense mechanism in EV patients.

The immune responses of the patients infected with various HPVs and the mode of wart regression depend on the HPV type. Most evident decrease in CMI responses in patients with warts was found to be associated with HPV3, HPV10 and HPV28- induced infections, and CMI mechanisms were shown to be involved in their regression.

1343 PAPILLOMAVIRUSES FROM EPIDERMODYSPLASIA VERRUCIFORMIS PATIENTS AND RENAL ALLOGRAFT RECIPIENTS, Herbert Pfister, Institut für Klinische Virologie, University Erlangen-Nürnberg, Erlangen, F.R. Germany

Patients with epidermodysplasia verruciformis (ev) suffer from congenital defects in cell-mediated immunity, which make them prone to infection with a large number of papillomavirus types, which are not regularly observed in the normal population. Individual patients are usually infected by a number of different virus types. From a representative case we isolated and cloned the DNAs of HPV5, 8, 19, 20, and 25 (1, 2). HPV5 predominated in the skin carcinoma. HPV8 DNA was also cloned from carcinoma DNA and represented a new variant, which differed from the prototype in several restriction enzyme cleavage sites mainly in the non-coding, regulatory region of the genome. The other DNAs were isolated from warts. The colinear genomes were aligned to define early and late regions and were partially sequenced. HPVs 8, 19, and 25 showed homology in different parts of their genomes.

It was repeatedly speculated if typical ev-viruses play a role in wart and cancer development in immunosuppressed transplant recipients because immune defects play an essential role in ev itself. A survey on 115 patients showed that ev specific viruses are not prevalent in transplant recipients. We detected HPV2 twice, HPV3 once, HPV4 and 10 twice,and HPV16 once.HPV2 (3) and16 could be demonstrated once each in cases of Bowen's disease of the skin and HPV16 once in a squamous cell carcinoma of the skin. This may indicate that these viruses play a role in malignant conversion of warts of immunosuppressed patients.

(1) Pfister et al., Cancer Res. 43, 1436-1441 (1983)
(2) Gassenmaier et al., J. Virol. 52, in press (1984)
(3) Pfister and Haneke, Arch. Dermatol. Res., 276, 123-125 (1984)

Papilloma Viruses in Human Oral and Laryngeal Diseases

1344 INTERFERON IN RECURRENT RESPIRATORY PAPILLOMATOSIS-A MULTICENTER CONTROLLED CLINICAL TRIAL. G.B. Healy, M.D., A.L. Trowbridge, R.N. Dept. of Otolaryngology, The Children's Hospital, Boston, MA 02115.

The objective of this national randomized controlled clinical trial is to evaluate the effectiveness of Human Leukocyte Interferon in combination with laser surgery versus laser surgery alone in the treatment of Pediatric Recurrent Respiratory Papillomatosis. Patient enrollment began in January of 1983 and ended in February of 1985. A total of 140 patients from 31 participating centers were enrolled with 70 randomized to receive Interferon therapy.

Human Leukocyte Interferon was administered intramuscularly at a dosage of $2mu/m^2$ daily for the first week then 3 times a week for 1 year to 50% of the patients randomized into the trial. Patients in both arms were evaluated endoscopically at entry, 1 month, 3 months, and then every 3 months for 2 years. At each endoscopy the extent of gross papilloma in the larynx and tracheobronchial tree was mapped pre-operatively and post-operatively according to 42 specific anatomical locations on detailed topgraphic data forms. The degree of pre-op and post-op obstruction in the larynx and/or tracheobronchial tree was documented in terms of percent airway obstruction.

Interferon's potential effect on the growth and development of children was also evaluated. Physical growth parameters were obtained on all patients at each visit and plotted by one individual on NCHS growth charts and computerized by percentiles. The developmental parameters were tabulated on special age appropriate developmental forms at 6 month intervals and reviewed by the Boston Children's Hospital Developmental Team.

In the first 22 months of the trial, 13 patients(25%) experienced some form of Interferon toxicity. Of these 13 patients, 7(14%) experienced LFT elevations, 5(10%) experienced transient neutropenia, and 1(1%) experienced general malaise, persistant fever, muscle aches and cramps, post injection. Of the 7 with elevated LFT's,2 remained stable on full dose after 1 alteration while 5 required frequent alterations, maintaining normal LFT's on half dose only. All 5 patients with transient neutropenia remained stable on full dose after 1 alteration. Two of the 5 neutropenias were most likely due to concurrent acute illnesses and not to the Interferon. No further toxicities have been reported in the last 6 months.

The degree of disease involvement, as reported on these data forms, will be serially evaluated by an objective group of statisticians to test if the extent of involvement at fixed points in time is different for the two treatment groups. The total number of sites involved, the location of new sites in relation to the original sites, contralateral disease and spread to adjacent anatomic areas will be considered. The primary analysis will center on time to relapse in relation to treatment and baseline data.

1345 CLINICAL AND EPIDEMIOLOGIC FEATURES OF RECURRENT RESPIRATORY PAPILLOMATOSIS, Haskins Kashima, M.D.*‡ Keerti Shah, M.D+and Brigid Leventhal, M.D.++
* Department of Otolaryngology, +Department of Immunology and Infectious Diseases, ++Department of Pediatric Oncology, Johns Hopkins Hospital, 600 N. Wolfe Street, Baltimore, MD 21205.

The term RRP is applied to histologically benign warty growths (papillomata) occurring in the upper respiratory tract and encompasses two clinical categories depending upon age at onset of disease. The juvenile onset form, commonly known as juvenile laryngeal papillomatosis, has onset prior to puberty and is characterized by papillomata occurring at multiple sites, tendency for regrowth after apparent complete excision and tumor growth by direct extension and implantation. The combination of rapid papilloma regrowth and relatively small size of airway necessitates frequent repeated operations and patients requiring a hundred or more endoscopies are not unusual. In the adult onset form, the papillomatous growths are usually solitary at initial presentation and successful treatment by complete excision is possible more often than in the juvenile onset cases. In both forms there is predilection for lesions to occur at squamo-columnar junctions.

It has been generally taught that JLP undergoes spontaneous regression at puberty; it may be more correct to conclude that the larger laryngo-trachea makes surgical instrumentation easier and eradication of lesions more complete. Spontaneous regression of respiratory papilloma is an uncommon occurrence. Malignant transformation of papilloma is uncommon and has been observed when papilloma has been irradiated.

Establishing the viral etiology of respiratory papillomatosis has been a recent and major achievement. HPV-types 6 and 11, associated with RRP, are also the etiologic agents identified with genital warts (condylomata). The latter condition, which is histologically similar ro respiratory papilloma, has been reported present at the time of delivery in a disproportionately large percentage of mothers giving birth to children who develop RRP. RRP patients at The Johns Hopkins Hospital have tended to be the first born of young (teenaged) mothers with genital warts. No siblings of RRP patients have been found to be afflicted with the disease. None of the sixty RRP patients (juvenile onset and adult onset) were born by Ceasarean section. Assuming the condyloma prevalence in women to be in the 1% to 2% range, the risk for developing RRP for a child born to an infected mother is crudely estimated to be between 1:100 and 1:1000.

The severity of papilloma whether judged by frequency or total number of operations, or by extent and nature of growths, varies from "mild" to "severe". Analysis of 22 patients

indicates that HPV-6c identifies the patient subgroup with more extensive disease. A severity score considering the number of anatomical sites diseased, the surface area involved and growth pattern, whether sessile or exophytic, has been developed. This scoring system is being utilized to evaluate the efficacy of adjuvant modalities for treatment of this condition.

1346 ANALYSIS OF HUMAN PAPILLOMAVIRUS IN THE RESPIRATORY AND GENITAL TRACTS DURING INTERFERON THERAPY. Phoebe Mounts,[1] Herbert H. Dedo,[2] Stanley A. Gall,[3] Haskins K. Kashima,[4] Brigid Leventhal,[4] George T. Singleton,[5] Phillip K. Weck,[6] and John K. Whisnant.[6] [1]The Johns Hopkins University, Baltimore, MD 21205. [2]University of California, San Francisco, CA 94143. [3]Duke University Medical Center, Durham, NC 27710. [4]The Johns Hopkins Hospital, Baltimore, MD 21205. [5]University of Florida, Gainesville, FL 32610. [6]Burroughs-Wellcome Company, Research Triangle Park, NC 27709.

We have been investigating the role of human papillomavirus type 6 (HPV-6) in the etiology, pathogenesis, and clinical manifestations of condyloma acuminata (genital warts) and respiratory papillomatosis. The papillomavirus genome is analyzed using the Southern transfer technology with DNA extracted from biopsy specimens of condyloma and respiratory papilloma. We have previously demonstrated the existence of multiple subtypes of HPV-6 which hybridize under stringent conditions but are distinguishable on the basis of restriction endonuclease digestion patterns. We have identified the HPV-6 subtype in papilloma specimens from patients registered in our multi-institutional clinical trials evaluating the efficacy of human lymphoblastoid interferon (Wellferon®) for condyloma and juvenile-onset respiratory papillomatosis. The relative frequency of occurrence of HPV-6 subtypes in biopsies from the respiratory and genital tracts is different. In the respiratory tract, HPV-6c was identified in 63% of the lesions, HPV-6d in 2%, HPV-6e in 15% and HPV-6f in 20%. In the genital tract, HPV-6c was identified in 8% of the lesions, HPV-6d in 8%, HPV-6e in 31%, and HPV-6f in 23%. There was no discernible geographical localization of the subtypes. We have found HPV-6c to be associated with more severe disease. HPV-6c was found in 82% of the respiratory papillomatosis patients who had a tracheostomy and in all of the patients who had pulmonary disease. There was no association between viral subtype and response to interferon.

1347 IN VITRO STUDIES OF LARYNGEAL PAPILLOMAS, Bettie M. Steinberg, Allan L. Abramson, Department of Otolaryngology and Communicative Disorders, Long Island Jewish-Hillside Medical Center, New Hyde Park, NY 11042.

We have developed a method for culturing epithelial cells in vitro from human laryngeal papillomas.[1] Using this technique, we have been able to compare the morphology, nutrient requirements and differentiation of papilloma cells and normal vocal cord epithelial cells.[2] The HPV DNA copy number is the same for cultured cells as the initial tissue biopsy,[2] confirming that the cultured cells are in fact derived from the papilloma and not from adjacent normal tissue.

The general morphology and appearance of the two cell types in culture is indistinguishable. However, there are differences in the distribution of actin and keratin fibers. Analysis of keratins by SDS-polyacrylamide gel electrophoresis shows variation in minor keratin bands for the two cell types; these are also seen in the original tissues. Addition of retinoids to serum free cultures enhanced the growth of both types of cells and prevented formation of cornified envelops. In vitro studies of the effects of interferon on these cells is in progress. Data to date suggests that thymidine incorporation is reduced equally in the two cell types, but that outgrowth from tissue fragments is not affected. Culture in the presence of interferon for 4-6 weeks does not effect HPV DNA copy numbers.

Problems and limitations to these studies, as well as plans for future experiments will be discussed.

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1348 INTERFERON TREATMENT OF LARYNGEAL PAPILLOMATOSIS: AN OVERVIEW, Hans Strander, Radiumhemmet, Karolinska Hospital, 104 01 Stockholm 60, Sweden.

There are three classes of human interferons (IFN) which have been characterized: alpha, beta, and gamma. The alpha interferons have been prepared either from leukocytes, (Le)IFN-α, lymphoblastoid cells, IFN-α(Ly), or from bacteria, rIFN-α2, rIFN-αA and rIFN-αD. Fibroblast IFNs have been prepared from tissue cultures, IFN-β, or from bacteria, rIFN-β. Gamma IFNs have been prepared either from natural sources, IFN-γ, or from bacteria, rIFN-γ. Several of these IFN preparations have been used to date in the treatment of juvenile laryngeal papillomatosis.

An overview of the litterature is given and an attempt is made to assemble recent results in the treatment of this disease with various IFN preparations. The results obtained so far can be summarized as follows: IFNs are able to cause regression, complete or partial, of juvenile laryngeal papillomatosis. On prolonged treatment it is possible in most patients to obtain a long disease free interval. It is not known whether patients can be cured from their disease by sole IFN treatment since the first patient was treated only 8 years ago. Optimal schedules and doses are not known but the lowest possible doses to achieve effects have been established. The mechanism of IFN action can only be suggested on the basis of previous clinical experience, biochemical investigations and animal experiments.

Summary: It is suggested that IFN treatment should be used on at least severe cases of juvenile laryngeal papillomatosis in combination with laser surgery.

Molecular Biology

1349 STRUCTURAL ANALYSIS OF BPV-1 mRNA's ISOLATED FROM BOVINE FIBROPAPILLOMAS, Carl C. Baker and Peter M. Howley, NCI, Bethesda, Maryland 2205

Bovine papilloma virus type 1 (BPV-1) causes cutaneous fibropapillomas in cattle and is tumorigenic when innoculated into hamsters. When BPV-1 DNA is transfected into mouse (C127) cells, only the transforming or "early" region is expressed and the cells are transformed, but no virus is produced. The mRNAs from transformed cells have been extensively analyzed by cDNA cloning amd sequence analysis (Y.-C. Yang, H. Okayama and P. Howley, PNAS, in press). Vegetative viral DNA synthesis, "late" transcription, capsid protein synthesis and virion assembly occur only in the differentiated keratinocytes of the papilloma. In order to study the transcription and function of the "late" viral genes, we have constructed a cDNA library with mRNA isolated from bovine fibropapillomas using the method of Okayama and Berg. Preliminary analysis of cDNA clones from the wart-specific region show that the polyadenylation (AATAAA) site at base 7175 is used. One species of mRNA has been identified which splices a portion of the E5 ORF in phase with the L1 ORF. Transcription of BPV-1 will be analyzed in nuclei isolated from BPV-1 transformed cells to determine the nature of the block to "late" gene expression. An attempt will be made to identify cellular and/or viral genes important in regulation of "late" gene transcription.

1350 STIMULATION OF $pp60^{c\text{-}src}$ TYROSYL KINASE ACTIVITY IN DNA TUMOR VIRUS TRANSFORMED CELLS, Joseph B. Bolen, Shorheh Amini and Virginia A. DeSeau, Laboratory of Tumor Virus Biology, NCI, Bethesda, Maryland 2205

We have examined the effect of DNA tumor virus transformation of rodent cells on the tyrosyl kinase activity of $pp60^{c\text{-}src}$. Previously, we found that $pp60^{c\text{-}src}$ kinase activity was stimulated in rodent cells transformed with polyoma virus following the association of the polyoma-encoded middle tumor antigen with $pp60^{c\text{-}src}$. Our present study demonstrates that rodent cells transformed by other DNA tumor viruses including Simian Virus 40, Adenovirus 2, Adenovirus 12, and Bovine Papillomavirus also can possess stimulated $pp60^{c\text{-}src}$ kinase activity. The increased levels of $pp60^{c\text{-}src}$ kinase activity in these transformed cells was not due to either src gene amplification or increased levels of src gene transcripts. Additionally, $pp60^{c\text{-}src}$ was not found to be physically associated with tumor antigens encoded by these viruses. These results show that $pp60^{c\text{-}src}$ kinase activation can occur in many different viral transformed cells and suggest that the mechanism of $pp60^{c\text{-}src}$ kinase stimulation differs from that observed in polyoma virus transformed cells.

1351 ELECTRON MICROSCOPICAL HETERODUPLEX COMPARISONS ON DNAs FROM HUMAN PAPILLOMA VIRUS TYPES 6, 11, 16 AND 18: Thomas R. Broker and Louise T. Chow, Biochemistry Department, P.O. Box 607, University of Rochester School of Medicine and Dentistry, 601 Elmwood Ave., Rochester, NY 14642.

Heteroduplexes between cloned HPV-6 and HPV-11 DNAs were prepared at low stringency (0.1 M Tris-Cl, 30% formamide, 22°C) and were examined by electron microscopy after spreading the samples in cytochrome c monolayers in the presence of different concentrations of formamide, to vary the effective stringency. The two DNAs are collinear and are of approximately the same length, in keeping with previous observations of many other pairs of human and animal papilloma viral DNAs. HPV-6 and -11 are very closely related and exhibit about 97% pairing in 30% formamide (T_m - 41°), 85% pairing in 50% formamide (T_m - 28°), and 50%-55% pairing in 60% formamide (T_m - 21°). DNA segments paired in the three preparations exceed 50%, 65% and 75% homology, respectively. The first genetic region to denature (in 30% formamide) was E5b. The second portions to denature (in 50% formamide) were E5a and intergenic region immediately downstream from L1. In 60% formamide, genetic regions E4, the 3' third of E1, and the 5' half of L2 began to denature in the heteroduplexes.

HPV-18 cloned by Boshart et al., was used to form heteroduplexes with HPV-6. They were paired over 25% to 43% of their lengths in 30% formamide, indicating these regions have at least 50% homology. Based on the DNA sequences of HPV-6 DNA, the major regions of genetic similarity between the viruses are in E1, L1, and both ends of E2. The viruses differ most in E6, E7, E2, E4, E5, the C-terminal two-thirds of L2, and the intergenic region.

Regions and degrees of homology and divergence in heteroduplexes between HPV-16 (cloned by Durst and collaborators) and HPV-6 closely resembled those between HPV-6 and -18. Surprisingly, therefore, heteroduplexes between HPV-16 and -18 exhibit less homology than either does to HPV-6. Additional heteroduplex studies of HPV-19 and -25 in collaboration with H. Pfister and of HPV-26 in collaboration with R. Ostrow are in progress.

1352 COMPARISON BETWEEN THE GENOMIC ORGANISATION OF HUMAN GENITAL PAPILLOMA VIRUSES, Klaus Dartmann, Elisabeth Schwarz, Lutz Gissman and Harald zur Hausen, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, 6900 Heidelberg, FRG

The large open reading frames of HPV 6 and 11 and the putative proteins derived from their nucleotide sequences were compared.
The overall nucleotide sequence homology between both types is 82 percent. At the protein level the homology varies between 58 percent (E5b) and 91 percent (E1).
The non-coding regulatory regions show at the nucleotide level only 10 percent of homologous sequences, suggesting that this region may play a role in possible differences of both virus types in host cell tropism.
The homology of the HPV 11 sequence to HPV 16 is much less pronounced (58 percent for the complete sequences).
The region of the putative early promoter contains a duplicated TATA-box-like sequence, separated by a direct repeat of a symmetric 12 bp element.
This structure is common to all four genital papilloma viruses HPV 6, 11, 16 and 18.

1353 THE NUCLEOTIDE SEQUENCE OF TWO VIRUS/CELL JUNCTION SITES IN A CERVICAL CARCINOMA AND THE ANALOGOUS REGION IN LYMPHOCYTE DNA, Matthias Dürst and Lutz Gissmann, Deutsches Krebsforschungszentrum, Institut für Virusforschung, Im Neuenheimer Feld 280, 6900 Heidelberg, FRG

The analysis of a number of malignant genital tumors suggests that at least some copies of the HPV16 or HPV18 genome are integrated within the host DNA.
In order to learn something about the mechanisms of integration two of the virus/cell junction fragments from a cervical carcinoma as well as the analogous region within the lymphocyte DNA of the same patient have been cloned and their nucleotide sequence is being determined.

1354 **ANALYSIS AND TRYPTIC PEPTIDE CHARACTERIZATION OF THE STRUCTURAL PROTEINS OF THE SHOPE (RABBIT) PAPILLOMA VIRUS.** Mark A. Feitelson[1], Felix O. Wettstein[2,3] and Jack G. Stevens[2]. [1]Division of Clinical Research, Fox Chase Cancer Center, Philadelphia, PA 19111; [2]Department of Microbiology and Immunology, UCLA School of Medicine; [3]Molecular Biology Institute, University of California, Los Angeles, CA 90024.

Purified Shope papilloma virus was radiolabelled by direct iodination using chloramine T or by indirect iodination (acylation) using the Bolton-Hunter reagent. Structural polypeptides were then characterized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and tryptic peptide mapping. SDS-PAGE of these preparations showed a major polypeptide at 67,000 daltons, 5-7 bands between 20,000 and 60,000 daltons, and 3 bands below 20,000 daltons in size which comigrated with calf thymus histones. The peptide map of the major polypeptide shared 45% homology with the intermediate size polypeptides (20,000-60,000 daltons), while the maps of these intermediate size components shared 70-75% homology among themselves. The results suggest that the Shope DNA possesses one gene coding for the major structural polypeptide and another coding for the family of intermediate size components. However, the apparent size of the major capsid polypeptide as well as its relationship to the intermediate size class of polypeptides suggest that some of its translated sequences are derived from the gene encoding these intermediate size components. These relationships could be the result of RNA splicing, post-translational modification, or both. Based upon this data, 40-50% of the Shope virus genome encodes the major and intermediate size structural polypeptides of the Shope papilloma virus.

1355 GENE EXPRESSION OF HPV IN CARCINOMA OF THE CERVIX, U. Karl Freese, Elisabeth Schwarz, Thomas Bauknecht and Harald zur Hausen, Deutsches Krebsforschungszentrum, 6900 Heidelberg, FRG

The presence of Human Papilloma Virus in cervical carcinomas and its gene expression have been studied. Analysis of DNA and RNA extracted from the same tumor biopsies revealed the concomittant presence of Human Papillomavirus DNA and RNA in the malignant tissues. The structure of these RNAs is presently under investigation. This work aims to further elucidate the genetic activity of Human Papilloma Viruses in cervical cancer as a step in defining the role of these viruses in the etiology of human neoplasia of the uterine cervix.

1356 AN OBLIGATORY TRANSFORMING FUNCTION OF BPV-1 IN THE E5 OPEN READING FRAME. D.E. Groff and W.D. Lancaster, Georgetown Univ. Med. Ctr., Washington, DC. 20007.

To define the functions of the bovine papillomavirus type (BPV-1) open reading frames (ORF) in cell transformation and autonomous viral DNA replication in transformed cells, the BPV-1 genome has been alterred at specific sites. The effect of these mutations were assayed by transformation of C127 and NIH3T3 mouse cells and the physical state of the viral DNA in transformed cells was determined. Mutants were generated by cleaving a BPV-1/pML2 shuttle vector with restriction enzymes, repairing the ends with T4 DNA polymerase or E. coli DNA polymerase I, Klenow fragment, and recircularizing with T4 DNA ligase. Resulting plasmids that were resistant to cleavage with the original enzyme were analyzed further. The E1 ORF was alterred at the EcoRI site (pos. 2118), the E2/3 and E4 ORFs at the KpnI site (pos. 3455), and the E5 ORF at the BstXI site (pos. 3889). The amino portion of the E2 ORF was alterred by deleting the NcoI fragment (pos. 2879-3089). The KpnI mutant transformed cells as well as wild-type DNA and the viral DNA remained episomal. The EcoRI mutant transformed cells at about 50% wild-type levels and the input DNA was exclusively high molecular weight and appeared to be integrated into host DNA. The NcoI mutant transformed cells at about 20% wild-type levels and the input DNA was high molecular weight but appeared to be catenated circles of various size as well as randomly integrated. The BstXI mutant failed to transform either C127 or NIH3T3 cells. Whether the BstXI alteration affects the translation of a viral gene product or a site on the BPV-1 DNA remains unclear.

1357 THE EFFECT OF POSITION ON GENE EXPRESSION IN BPV VECTORS, Nava Sarver, Ruth Muschel, Janet C. Byrne, George Khoury, and Peter M. Howley. NCI, Bethesda, Maryland 20205
The effect of the site of insertion on foreign gene expression in a BPV vector was assessed using the rat preproinsulin gene (rIl). Using derivatives of the pdBPV-1 (142-6) vector which consists of the BamHI linear genome of BPV-1 DNA cloned into pML2d, the rIl gene was inserted at each of the BPV-1/pML2 junctions in either transcriptional orientation. Transformed lines of C127 cells were established and assayed for rIl expression. Cells containing the rIl gene at the 3' end of the transforming region made rat proinsulin whereas cells with the gene at the 5' end of the non-transforming region did not. There were no differences in plasmid copy number or in the extent of rearrangement which could account for this difference. We conclude that the expression of the rat preproinsulin gene (which is normally tissue specific for pancreatic islet cells) is due to the transcripitonal activation afforded by viral "enhancer" sequences located at the 3' end of the transforming region. The rat preproinsulin gene located in the "blocked" position not adjacent to the "enhancer" could be transcriptionally activated by the adjacent insertion of a DNA fragment containing the SV40, MSV, or BPV enhancer. Thus, a gene which is normally expressed in a cell specific manner may be expressed in a BPV vector when placed adjacent to a viral enhancer. Intervening BPV or pML2 sequences block this enhancer mediated activation.

1358 Expression of Biologically Active Fertility Hormones in a Bovine Papilloma Virus System

Nancy Hsiung, Barbara Fleming, Vinnie Velluci and Toni Beck - Integrated Genetics, Inc. 31 New York Avenue, Framingham, MA 01701

The human glycoprotein hormones are encoded by a family of related genes. Luteinizing (LH) and follicle stimulating (FSH) hormones are synthesized in the anterior pituitary gland and are involved in the regulation of the development and activity of the gonads. Both hormones are dimers consisting of a common alpha subunit and a beta subunit which confers the specificity. Both LH and FSH genes have been cloned and inserted into a variety of Bovine Papilloma Virus vectors for expression in the mouse host cells. Foci were isolated and clones producing and secreting dimeric LH or FSH were identified. The resulting stable transformants from different vectors were characterized and a comparison of the production levels was obtained. Studies on protein structure and its relation to functions are described.

1359 GENERAL METHOD FOR IDENTIFYING SEQUENCE SPECIFIC DNA BINDING PROTEINS, David Levens, Barbara A. Spalholz and Peter M. Howley, NCI, Bethesda, Maryland 20205
We have developed a general method for the identification of sequence specific DNA binding proteins. A well-characterized protein-DNA interaction is used as a hook to fish out proteins recognizing specific sequences from crude extracts or fractions thereof. The sequence of interest is cloned adjacent to the lac operator and the resulting plasmid is incubated with a lac repressor-beta-galactosidase fusion protein which retains full operator and inducer binding properties. The DNA fragment is precipitated by the addition of Immunobeads with covalently bound goat anti-rabbit antibodies which have been saturated with affinity purified rabbit anti-beta-galactosidase antibodies. This forms an affinity matrix for the unknown protein interacting with the regulatory sequence of interest. When incubated with extracts containing the unknown protein and with excess competitor DNA, the specific protein can be cleanly precipitated. Upon addition of IPTG, a protein-DNA complex composed of the specific restriction fragment and the unknown protein is released permitting the analysis of the unknown proteins by conventional methods.

We have tested this method using crude preparations of the yeast mitochondrial RNA polymerase and have identified a 70,000MW peptide which binds to the promoter region of the yeast mitochondrial 14S rRNA gene. We have correlated the presence of this protein with the ability to support faithful transcription in vitro from the 14S rRNA promoter.

1360 EXPRESSION OF OPEN READING FRAME SEQUENCES FROM THE EARLY REGIONS OF BPV AND HPV, Robert Mallon and Vittorio Defendi, NYU Medical Center, New York, NY 10016

To gain insight into the mechanism of papilloma virus replication and also papilloma induced cellular transformation we are expressing papilloma virus early region open reading frame sequences in Escherichia coli. We are making use of a modified prokaryotic expression vector, pRW10, that incorporates the pL promoter of bacteriophage λ and the CII translation start signals (W. Sisk, D. Court, personal communication). Also contained on this vector is the polylinker region of the M13 mp19 virus and the lac Z gene. Vector organization (pL-CII-polylinker-lac Z) allows for insertion of papilloma sequences with either sticky or blunt ends. Blunt end papilloma fragments (generated by DNAse I or Bal 31 treatment) that contain open reading frames when inserted into this vector can establish a continuous read through into the lac Z gene resulting in a lac Z+ blue colony when plated on LB/amp plates containing X-gal.

We are using this system to express the E2 and other open reading frames from both HPV6b and BPVI. These proteins will be used to generate antibodies to probe papilloma transformed cell lines. (Supported by NIH grants #CA 16239 and #CA 09161).

1361

DNA SEQUENCE AND GENOME ORGANIZATION OF BOVINE PAPILLOMAVIRUS TYPE-4 (BPV-4)

Kamalesh R. Patel, Kenneth T. Smith and M. Saveria Campo, The Beatson Institute for Cancer Research, Glasgow G61 1BD.

Sequence analysis of the DNA of BPV-4 has revealed that the arrangement of the genes is similar to that identified in other Papillomaviruses. Only one of the strands is transcribed, whilst the other strand contains numerous stop codons. Comparative analysis of the amino acid sequences of the major open reading frames with other Papillomaviruses has localised the 'Early' and 'Late' genes. The L1 and L2 genes are separated by an intragenic region which contains two promoter-like sequences with CAAT, TATAA, and ACA consensus domains. An open reading frame designated as E2 has been identified at the 5' end of L2. Computer-aided sequence homology searches have identified enhancer-like and PMS-like (Plasmid Maintenance Sequence) elements in the L2 open reading frame. The sequence data is in concordance with the S1 nuclease mapping of virus-specific RNA transcripts in productive warts. Further corroborative evidence has been obtained by DNA sequencing of BPV4-specific cDNA molecules.

1362 A VARIENT OF HUMAN PAPILLOMAVIRUS TYPE-6 CLONED FROM A RAPIDLY GROWING VERRUCOUS CARCINOMA, Robert F. Rando and Wayne D. Lancaster, Georgetown University, Washington, D.C. 20007

DNA sequences of a human papillomavirus (HPV) were detected in a rapidly growing vulvar verrucous carcinoma when probed under stringent hybridization conditions using HPV-6b DNA as a probe. These sequences (HPV-vc) were cloned and the DNA genome compared to that of HPV-6b for sequence homology. Saturation as well as Southern blot hybridization analysis under stringent conditions indicated no detectable differences in sequence homology. However, analysis of restriction endonuclease cleavage patterns and subsequent DNA sequencing demonstrated an additional 106 nucleotides in the HPV-vc DNA located in the non-coding region of HPV-6b which contains the putative replication and early gene transcriptional control elements. Two small inserts (19 and 15 bases) contain sequences not found in HPV-6b and demonstrate a striking homology to regions of human interferon genes. Another alteration in the HPV-vc genome is a 74 nucleotide addition in the purine-thymidine rich region 3' - to the end of the L1 reading frame. This insert is homologous to the virus DNA sequences 5' - to this insert. In, addition parts of this insert are homologous to conserved G/T rich repeated sequences in human DNA and regions in the human cardiac muscle actin gene.

1363 DETECTION OF PAPILLOMAVIRUS GENOMES AND EVIDENCE FOR AMPLIFICATION OF THE ONCOGENES c-myc AND c-Ha-ras IN CARCINOMAS OF THE UTERINE CERVIX, Guy F. Riou, Michel Barrois, Isabelle Tordjman, Viviane Dutronquay* and Gérard Orth*, Institut Gustave Roussy, 94800 Villejuif and *Institut Pasteur, Paris, France.

Invasive squamous cell carcinomas of the uterine cervix from 26 untreated patients were examined for the presence of human papillomavirus (HPV) genomes and for the state of the oncogenes c-myc and c-Ha-ras. Blot hybridization experiments have demonstrated the presence of the genome of HPV 16 in 12 tumors, of HPV18 in 2 tumors and that of the genomes of HPV types weakly related to HPV 16 or HPV 18 in 7 others. In 15 of the 18 tumors corresponding to advanced stages of the disease (stages 3 and 4) there was a 3-30 fold amplification of c-myc and in 10 tumors an 6-30 fold amplification of c-Ha-ras. A concomitant amplification of both oncogenes was found in 10 cancers. The oncogene c-myc or c-Ha-ras was only weakly amplified in 2 of the 7 tumors confined to the cervix (stage 1). Neither HPV DNA sequences nor oncogene amplification were detected in the leukocytes of 12 of these patients and in 2 specimens of normal uterine cervix. These data extend previous results* which confirmed the role of specific HPV types in the development of cervical carcinomas and suggested that cellular oncogenes, activated through an amplification process are involved in at least some steps of tumor progression.

*(G. Riou, M. Barrois, I. Tordjman, V. Dutronquay et G. Orth, C. R. Acad. Sc. Paris, 1984, in press).

1364 THE NUCLEOTIDE SEQUENCE OF HUMAN PAPILLOMA VIRUS TYPE 16: EXPRESSION OF OPEN READING FRAMES IN E.COLI, Walter Röwekamp and Klaus Seedorf, Institute of Cell and Tumor Biology, German Cancer Research Center, Im Neuenheimer Feld 280, D - 6900 Heidelberg.

Human papillomavirus type 16 (HPV16) DNA has been found in biopsy materials from cervical, vulval and penile cancers. The data indicate that HPV16 DNA prevails in malignant tumors (1, 2).

We have determined the nucleotide sequence of the HPV16 genome. The size and organization of the open reading frames is similar to that of other papilloma DNAs already sequenced. These data prove that HPV16 represents a new member of the HPV family (3). We have initiated experiments to express all possible open reading frames in E.coli in order to raise antibodies against different virus proteins.

1. Dürst, M. et. al. (1983) PNAS 80, 3812-3815.
2. Ibenberg, H. et. al. (1983) Int. J. Cancer 32, 563-565.
3. Seedorf, K. and Röwekamp, W.G. (in preparation)

1365 STABLE HIGH-LEVEL EXPRESSION OF HUMAN γ-INTERFERON USING A BPV VECTOR. Nava Sarver, Margret Hood and John Link. Biotechnology Research Center/Revlon, 6715 Electronic Drive, Springfield, VA 22151.

We have obtained a sustained high-level expression of human γ-interferon (γ-IFN) cDNA cloned in a BPV shuttle vector. Analysis of gene expression in several C127-transformed foci generated with the recombinant molecule indicated considerable heterogeneity in the levels of γ-IFN secreted into the medium. In contrast, single-cell clones exhibited a uniform level of expression similar to that of the original parental line. Southern DNA analysis of transformants expressing high levels of γ-IFN ($>10^5$U/ml/10^6 cells/24 hrs) revealed that the transferred DNA is maintained as an episome and for the most part is unrearranged. However, high frequency of molecular rearrangements, including excision of the IFN cDNA segment, were observed when the input DNA contained duplicated fragments.

Polyacrylamide gel electrophoresis of intra- and extracellular proteins demonstrate the presence of a homogeneous peptide comigrating with natural γ-IFN marker. Crude preparation of the recombinant-derived protein (a) confers protection against EMC infection of WISH cells, (b) is neutralized by monoclonal antibodies against γ-IFN, (c) competes with natural γ-IFN in an immunoprecipitation reaction, and (d) induces the expression of class II major histocompatibility complex antigens in fibroblasts and in endothelial cells. Stability tests indicate that the protein is stable in the medium for up to five days.

1366 IDENTIFICATION OF A SECOND TRANSFORMING GENE IN BOVINE PAPILOMAVIRUS DNA. John T. Schiller, William C. Vass, Elliot J. Androphy, and Douglas R. Lowy, Laboratory of Cellular Oncology, National Institutes of Health, Bethesda, MD 20205.
Previous studies of Bovine Papillomavirus (BPV-1) induced transformation have found that a cloned 5.4 kilobase fragment (69T) of the genome is transforming and that a 2.3 kb segment from the 3' end of this fragment is also transforming if activated by a retroviral regulatory element (the long terminal repeat). We now report that 69T contains another transforming segment near its 5' end. Since this second segment does not overlap the 3' transforming segment, we conclude that BPV-1 encodes at least two genes that can independently transform cultured cells. Mutational analysis of the 5' transforming segment suggests that the transforming gene of this segment lies within the E6 open reading frame (ORF). The two transforming segments differ in their biological activity in that the E6 containing fragment can transform C127 but not NIH3T3 mouse cells whereas the 3' fragment can transform both cell lines. E6 transformed C127 cells are fully transformed in that they form colonies in soft agar and tumors in nude mice.

1367 TRANS-ACTIVATION OF A BPV-1 ENHANCER, Barbara A. Spalholz, Yu-Chung Yang and Peter M. Howley, Laboratory of Tumor Virus Biology, NCI, Bethesda, Maryland 20205
We have cloned fragments of the bovine papilloma virus (BPV-1) genome into the plasmid vector $pA_{10}CAT$, which contains the gene for chloramphenicol acetyl transferase (CAT) directed by the SV40 enhancer-deleted early promoter. From the analysis of the CAT activity in cells transfected with the BPV-1/$pA_{10}CAT$ recombinant plasmids, we have mapped a transcriptional activator sequence within the 1.0 kb (HindIII to Hpa I) non-coding region of the BPV-1 genome. This fragment exhibits enhancer activity when inserted either 5' or 3' to the CAT gene and this activity is independent of the orientation of the inserted fragment. The level of CAT activity directed by this enhancer is significantly augmented in BPV-1 transformed C127 cells as compared to untransformed C127 cells, suggesting that a viral gene product may be responsible for this trans-activation. Using defined deletion mutants of BPV-1 DNA and viral cDNAs expressed off of an SV40 early promoter, we have shown that the expression of this trans-activation function maps to the 3' ORFs of the transforming region. Further studies to specifically delineate this sequence in the BPV-1 genome and to map the viral gene responsible for the expression of this trans-activation are in progress. A previous enhancer segment mapped by Lusky et al. (Mol. Cell. Biol. 3:1108, 1983) is unaffected by this trans-activation function.

1368 TRANS-ACTING FUNCTIONS AND CIS-LINKED SEQUENCES REGULATING PAPILLOMA VIRUS GENE EXPRESSION. T.Haugen, G.Ginder, L.Hattig, L.Cooling, and L.Turek, VAMC and University of Iowa College of Medicine, Iowa City, IA 52240.
To identify trans-acting regulatory BPV gene(s) and to assess their role in tissue-dependent autoregulation of BPV expression, we constructed two sets of chimeric vectors: (i) expression vectors designed to express predicted peptide-coding regions of the BPV genome; and (ii) target vectors in which detectable expression of prokaryotic genes (either CAT or neo) depended on the cis-acting enhancer/activator potential of a BPV segment. Activity of the target vector gene in transfected mouse cells was determined alone or in cotransfection with BPV, expression vectors and selected BPV mutants. A previously described activator sequence at the downstream end of the BPV early region (bases 3838-4451) exhibited a weak cis activity in transient expression experiments, and was not further stimulated by cotransfected expression vectors. A fragment of the upstream non-coding region between bases 6835-7279 acted as a relatively weak cis-enhancer. Its action was increased dramatically by a trans-acting product of early genes between bases 1-945 (the E6-E7 open reading frames). When additional BPV DNA bases 7279-7477 were contiguous to this cis element, its enhancer function was partially suppressed. Another cis element whose activity was greatly increased by a BPV-encoded trans function was found in a fragment spanning bases 6276-6946 of the BPV late region. We conclude that BPV encodes at least one trans-acting autoregulatory function in the E6-E7 region. Experiments are underway to determine which of the putative peptide products of the E6-E7 region is responsible for the trans effect by using expression vectors with frameshift mutations, termination codons, and plasmids expressing processed cDNAs.

1369 DELETION MUTANTS IN THE BOVINE PAPILLOMA VIRUS: EFFECTS ON CELLULAR TRANSFORMATION, Karl V. Voelkerding and Jean Spence, University of Utah, S.L.C., Utah 84132

Our experiments were designed to develop and characterize a BPV-1 shuttle vector containing a selectable marker and to use this vector system to introduce deletion mutants in the viral genome to assay for their effect(s) on cellular transformation. The plasmid p140BPV was constructed by cloning the entire BPV-1 genome into pRMH140, a pBR322 based vector which contains the Neo^r gene of Tn5 under the transcriptional control of the ASV LTR. The vector p140BPV, when introduced into C127 cells, confers G418 resistance and results in morphological transformation with high efficiency. Southern blot analysis demonstrates that p140BPV exists predominantly as a multicopy, autonomous plasmid. We constructed two mutants; p140BPV-A has viral sequences deleted between the NcoI site at base 2878 and the NcoI site at base 3039, removing only E2 open reading frame sequences; p140BPV-C has viral sequences deleted between the BstEII site at 2405 and the BstXI site at 3881. In transformation experiments both p140BPV-A and p140BPV-C conferred resistance to G418 but their efficiencies of of morphological transformation of C127 cells were 10% and less than 5%, respectively, as compared to p140BPV which contains the intact viral genome. Analysis by Southern blot indicates that p140BPV-C integrates into the host genome. As both mutants contain deletions involving the E2 open reading frame, and p140BPVAis deleted only in E2 sequences, these results implicate the putative E2 protein as critical for cellular transformation.

1370 LOCALIZATION OF HPV-5 TRANSFORMING FUNCTIONS, Susan L. Watts*, Louise T. Chow*, Ronald S. Ostrow#, Anthony J. Faras#, and Thomas R. Broker*; *Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724 and #Department of Microbiology, University of Minnesota, Minneapolis, MN 55455 USA

We have shown that HPV-5 DNA can morphologically transform mouse C127 cells in culture. Based on the common DNA sequence organization of all human and animal papillomaviruses sequenced thus far and on electron microscopic heteroduplex analysis indicating regions of homology between the HPV-5 genome and the sequenced genome of HPV-1, we have determined the approximate locations of the putative open translation frames of the HPV-5 genome. Naturally-occurring mutant HPV-5 DNAs isolated from carcinomas of epidermodysplasia verruciformis patients represent deletions within the proposed late region of the virus genome yet retain the abilities to transform C127 cells and to replicate as persistent episomes within the transformed cells. Subgenomic fragments corresponding to portions of the proposed early genomic region also transform cultured cells but integrate into the host cell genome. Vectors containing these fragments as well as mutants containing defined deletions within the early region are being used to further localize the HPV-5 transforming functions.

1371 FUNCTIONAL ANALYSIS OF VIRAL cDNAs FROM BPV-1 TRANSFORMED CELLS, Yu-Chung Yang, Janet C. Byrne, and Peter M. Howley. Laboratory of Tumor Virus Biology, NCI Bethesda, Maryland 20205.

We have previously constructed a cDNA library from mRNA of BPV-1 transformed mouse cells using an Okayama-Berg expression vector (Yang, Okayama, and Howley, PNAS, 1985, in press). This library contained seven classes representative of RNA species which are generated by differential splicing and which have a common 3' end. In conjunction with the open reading frames (ORFs) for BPV-1, it is possible to predict the structure of the potential encoded proteins. Two distinct classes of cDNA molecules can each independently transform mouse C127 when transcribed from the SV40 early promoter. One class contains the E6 ORF intact and the other class contains the E2 ORF intact. Since other downstream ORFs which could potentially be translated are also contained in each of these classes of cDNA molecules, linker fragments containing translational termination codons were introduced into each of the potential ORFs of these cDNAs to permit a more precise localization of the viral transforming functions. The results of these studies will be presented.

Immunology and Therapeutic Approaches

1372 PAPILLOMAS: CURRENT TREATMENT IN THE LARYNX, TRACHEA, PHARYNX AND NOSE, Herbert H. Dedo, Department of Otolaryngology, University of California, San Francisco 94143.

We still do not have a good treatment for papillomas in the respiratory tract and no effective treatment at all for the papillomas in the lung parenchyma at the present time. Prior to the development of laryngeal surgical instruments patients lost their ability to speak and then were asphyxiated. Perhaps 5-10% required tracheotomies and even multiple surgical procedures rarely cured them. What is needed is a chemical means of preventing or curing the virus infection which causes papilloma. While such a vaccine or antibiotic is being developed the best method has continued to be repeated surgical removal of these masses. The latest major advance appeared in the early 1970s with the CO2 laser for removal of laryngeal papillomas. Improved models have dramatically improved the thoroughness and precision with which we can remove these papillomas by vaporizing them away with an unexpected side benefit that the heat of vaporization seals the capillaries in the epithelium and submucosal tissues improving the operative field. The CO2 laser is now making it possible to remove papillomas all the way from the anterior nares deep into the mainstem bronchi. However, we still can not effectively remove or treat papillomas in the parenchyma of the lung.

Intraoperative photographs show the improvement in the appearance of the larynx of some of these patients being treated with modern CO2 laser via microdirect laryngoscopy, pharyngoscopy, nasoscopy, and bronchoscopy followed by painting the area of removal with podophyllum to minimize the risk of reimplantation of more viral particles. In resistant cases, alpha interferon is being used to improve suppression.

In summary, current surgical techniques are providing dramatically better results in the treatment of airway papillomatosis than those achieved in the past but some patients do not seem to have proper tissue resistance or some viral strains are more vigorous than others. It still requires too many surgical procedures in many patients who are not able to be cured of papilloma so there is a real need for a chemical cure for this disorder.

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1373 THERAPY OF RESISTANT CONDYLOMA ACUMINATA WITH NYMPHOBLASTOID INTERFERON. Stanley A. Gall, Department of Obstetrics and Gynecology, University of Illinois at Chicago, College of Medicine, Chicago, IL 60612; Connie E. Hughes, R.N., Duke University Medical Center, Durham, NC; John Whisnant and Philip Weck, Burroughs-Wellcome Co., Research Triangle Park, NC.

Condyloma acuminata or genital warts, are verrucous growths frequently found in sexually active females and males. Modes of therapy include Keratolytic agents such as podophyllin, trichoracetic acid or 5 fluoro-uracil with recurrent disease treated with cryotherapy surgical excession or laser. Approximately 5% of patient will have persistent condyloma despite current therapy. Our present study was designed to treat patients who failed conventional therapy and to evaluate the efficacy, toxicity and tolerability of Intramuscular and intralesional administration of Wellferon (lymphoblastoid Interferon). Trials were conducted at Duke University Medical Center. Patients were required to have condyloma present for at least 6 months and received at least ten Podophyllin treatments. Patients were evaluated with bidimensional measurements of each wart on a divided grid drawing, photographs, biopsies for histologic evaluation and DNA typing. Blood was obtained for hematological and Immunological testing. Interferon was administered I.M. at $5MU/M^2$ daily for 28 days then three times a week for two additional weeks. Evaluations were done weekly for four weeks then every other week. Patients with total disappearance condyloma were considered complete responders (CR) while a partial response was a 50% or greater decrease in wart's size and no response was no change or less than 50% decrease in size. Patients who were CR were observed 6 months; those who were PR continued to receive Interferon three times per week for 6 weeks and if the patient was NR, intralesional therapy was offered. Complete blood count, liver and renal tests were obtained. Seventeen female patients were entered on this study with mean age 31.2 and range 18-48 years and mean duration of disease of 2.5 years with 14.1 prior podophyllin treatments. Sixteen of seventeen patients were evaluable with 8/16 (50%) CR; 7/16 (44%) PR and 1/16 (6%) NR after 6 weeks of therapy. Three patients who were PR became CR with intralesional therapy, therefore 11/16 (69%) patients treated with Wellferon were judged CR at protocal's end. The dynamics of lesion regression shows little objective response for the first 14 days of therapy but the majority of wart regression occurred in the next 3-4 weeks. Biological side effects occurred in all patients but gradually decreased with time and especially when three times a week administration. Neutrophils, eosinophils, WBC and platelets were significantly decreased from normal on all days but no patient experienced clinical infection or bleeding diathesis. T

helper/T suppresor ratios, mitogen stimulation of lymphocytes and IgG and IgA concentrations were normal. However IgM concentrations were elevated, endogenous interferon was present and 4/14 (29%) patients were DNCB negative. Wellferon seems to be efficacious in the treatment of women with resistant and persistent condyloma acuminata.

1374 CYTOTOXIC AND ANTIVIRAL DRUGS FOR THE TREATMENT OF PAPILLOMATOSIS, Brigid G. Leventhal and Haskins K. Kashima, Johns Hopkins Hospital, Baltimore, MD 21205. There are a number of problems in the treatment of papillomas. First: the lesions are difficult to quantitate so that response, if it is less than complete, is hard to define. Second, occasional lesions undergo spontaneous regression so trials should be controlled, but patient numbers are small. Third, occasional lesions undergo malignant degeneration and this tendency is exaggerated in the face of known carcinogens such as radiation and cigarette smoke: any cytotoxic drug might then serve as a cocarcinogen. For all these reasons a risk benefit ratio for therapy is hard to construct. Nevertheless there are a number of therapeutic maneuvers which have been attempted. Papillomas are caused by DNA viruses which replicate in differentiating epithelial cells. In order to completely eliminate viral lesions, it is probably necessary to remove the entire epithelium or to treat the patient systemically so that a therapeutic level of drug is achieved in all epithelial layers. The fact that tissue destruction at any desired depth can be achieved with lasers and that surgery has failed effectively to eradicate disease at least in the larynx makes one fear that local therapy may not result in optimal disease control. Additional topical therapy has been attempted. Local podophyllum has resulted in regression of some lesions, usually associated with an inflammatory response. Systemic toxicity and possible induction of dysplasia has been reported. Antimetabolites, specifically IUdR and 5 FU have been reported to be successful when applied locally. The DNA intercalator, bleomycin; the alkylating agent, thioTEPA; the mitotic inhibitor, colchicine; and retinoids to induce epithelial cell differentiation have all been tried topically with occasional success. Similar agents have all been tried for systemic therapy. Antimetabolites such as methotrexate and 5 FU have caused regression of lesions. One patient with testicular cancer had complete regression of incidental papillomas with one course of bleomycin, velban and cisplatinum. Another patient, with extensive bronchial papillomas, responded to bleomycin alone. The artificial interferon inducer Poly ICLC caused a response in 2 patients, but other "antiviral" agents/besides interferon have either not been tried or not been effective. Nonspecific immune stimulants such as levamisole or cymetidine which inhibits suppressor cell activity have not been active when tried alone. All these trials have involved small numbers of patients. It seems that destruction of the obviously involved epithelial cells themselves has not worked. Interferon alone has some effect and compounds that enhance either the antiviral or antiproliferative effect of interferon should be tried. No specific molecular biologic or immunologic characteristics of the virus have as yet been identified which would allow us to selectively pick agents which, for example, inhibit viral DNA replication without affecting that of the host cell, but knowledge in this area is advancing so rapidly that this should be seen as a real possibility for the future.

1375 RESPONSE OF HUMAN PAPILLOMA-ASSOCIATED DISEASES TO RETINOIDS (vitamin A derivatives), F.L. Meyskens, Jr., E.A. Surwit, D.S. Alberts, and N.S. Levine, Depts of Medicine and Ob/Gyn, University of Arizona and Arizona Cancer Center, Tucson, AZ, 85724.

We have investigated the effect of retinoids on HPV-associated diseases, including cervical dysplasia (CD), laryngeal papillomatosis, (LP), and epidermal dysplasia verruciformis(EDV). For patients with CD we have used a cervical cap with an inert collagen sponge insert to deliver B-transretinoic acid (tRA, vitamin A acid); the cap is changed daily for a total of 4 days. We conducted a phase I trial and determined that a concentration of 0.37% tRA was safe for phase II efficacy trials (1). Patients received the initial treatment and were retreated every 3 months for 2 days for 9 months. They were evaluated before each course by colposcopy, cytology, and if both were negative, by biopsy. Six months after therapy had been stopped, 18 patients were evaluable for response. Complete responses (CR) were shown in 8 and partial responses (PR) in 5 patients. A phase III randomized trial against placebo is planned. We have also treated 5 patients with refractory LP (2) using oral 13-cis retinoic acid (13cRA). Three sustained CR were obtained. Using an ethyl ester derivative of retinoic acid (Etretinate) Bichler has reported CR in 28 of 42 patients with LP (3). We have also documented a PR in a patient with severe EDV using oral 13cRA (4). Others have also documented response of EDV to retinoids (4). Of interest is the response of patients with mycosis fungoides (MF), a T-cell cutaneous lymphoma, to oral retinoids. Although HTLV has been classically associated with a clinically different type of T-cell lymphoma, evidence for its presence has also been suggested in MF. We and others have demonstrated marked sensitivity of this disease to 13cRA and Etretinate with excellent responses in 50% of patients (5-8). The responsiveness of several viral associated premalignant conditions to retinoids is of considerable interest and suggests that use of these compounds may provide a new approach to prevention and treatment as well as suggesting a way to probe the biology of these diseases.

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1376 HIGH LEVEL EXPRESSION OF THE BPV-1 E1, E2, L1 and L2 OPEN READING FRAMES AND CLINICAL STUDIES ON THE EFFECTIVENESS OF AN L1 SUBUNIT VACCINE, William Pilacinski, Kimberly Glassman, David Reed, Melissa Lum, Molecular Genetics, Inc., Minnetonka, MN 55343 and Donald L. Glassman, College of Veterinary Medicine, University of Minnesota, St. Paul, MN 55108.

We had previously reported the cloning and expression in E. coli of the BPV-1 L1 and L2 open reading frames together with the results from some immunological studies on the bacterial expression products (1). Further work has included attempts to clone the other two major open reading frames, E1 and E2, the use of higher level E. coli promoters and the initiation of clinical studies on the use of the L1 product as a subunit vaccine in cattle.

Expression in E. coli by the lac promoter of the naturally terminating BPV-1 open reading frames results in the low level expression of a product which cannot be easily detected on Coomassie stained polyacrylamide protein gels. As reported with other proteins (2, 3), the carboxy-end fusion of a BPV-1 open reading frame to the E. coli β-galactosidase (β-gal) gene results in the expression of a stable protein that accumulates in the cell as an insoluble aggregate. This aggregation also occurs with the naturally terminating open reading frame when the level of expression is increased by the substitution of a higher level promoter, in this case an E. coli trp-lac promoter-operator hybrid. This level of expression is lethal or extremely deleterious to the cell and expression must be controlled to maintain the plasmid within E. coli.

A preliminary study with a small number of animals suggested that the L1::β-gal fusion product may be useful for the prevention of BPV-1 induced warts in cattle. Studies with greater numbers of animals, testing both the prophylactic and therapeutic usefulness of the L1 product are presently in progress.

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Transcription

1377 SYNTHESIS OF HPV mRNAS FROM HEAT SHOCK AND SV40 EXPRESSION VECTORS AND CHARACTERIZATION OF THE COMPLEX FAMILIES OF RNAS BY EM ANALYSIS OF RNA:DNA HETERODUPLEXES: Louise T. Chow, Anthony J. Pelletier, Ute Brinckmann, Shinta Cheng, and Thomas R. Broker, Biochemistry Department, P.O. Box 607, University of Rochester School of Medicine and Dentistry, 601 Elmwood Avenue, Rochester, New York 14642.

We have assembled a vector which consists of modified pML2, the SV40 origin of replicatio and enhancer sequences, and the promoter for the Drosophila gene encoding the heat shock protein hsp 70. Other vectors have either the SV40 early or the SV40 late promoter instead of the hsp 70 promoter. HPV-1 or HPV-6 DNAs have been opened immediately upstream of the E-region and cloned adjacent to the surrogate promoter so that transcription would be expected to start within the HPV sequences. Forty hours after transfection of the hsp 70-containing recombinant DNA into COS A2 cells and amplification of the plasmid DNAs, the cells were shocked at 43°C. The recovered cytoplasmic mRNAs were analysed by electron microscopical heteroduplex methods. The hsp-HPV clone gave about 100 fold more HPV-1 specific mRNA than had been observed previously. Most of the many mRNA species were indeed derived from the Drosophila heat shock promoter. All contained one or more splices. The most abundant spans the overlapping open translation frames E4 and the C-terminal half of E2 in the transformation region. Less abundant species cover the L1 region coding for the major capsid protein. Minor species have sequences that span the E6, E7 and E4 regions or that extend through the E1 and L2 regions. Transfection of COS cells with clones containing the SV40 early promoter also produced a large amount of HPV-specific transcripts. Interestingly, despite the different promoters and 5' cap sites, the main bodies of the messages and the internal RNA splice sites are the same in both kinds of RNA preparations.

We have transformed mouse C127 , Rat 4, mouse L tk$^-$, human 143 tk$^-$ cells and rat primary kidney cells with a variety of cloned HPV-1 and HPV-6 DNAs, of which several also include in the vector the HSV tk gene or the neomycin resistance gene from bacterial transposon Tn5. HAT- or G418-resistant cells as well as morphological transformants were picked after 2-3 weeks. Most of the transformants contain very low copy number of the input DNA in an integrated state. In this respect, HPV behaves differently from bovine papilloma virus or viral vectors. We could not detect any HPV mRNA in these transformants.

1378 MESSENGER RNAs FROM THE TRANSFORMING REGION OF BOVINE PAPILLOMAVIRUS TYPE I, Ulf Pettersson, Arne Stenlund, Jan Zabielski, Harri Ahola, Mart Ustav, Jorge Moreno-Lopez[1], Departments of Medical Genetics and Microbiology, Biomedical Center, Box 589, S-751 23 Uppsala, Sweden, [1]Department of Veterinary Virology, Biomedical Center, Box 585, S-751 23 Uppsala, Sweden

mRNAs present in C127 mouse cells transformed by bovine papillomavirus type 1 (BPV-1) were studied by the S1 nuclease protection technique, Northern blotting, and electron microscopic heteroduplex analysis. The results revealed at least five classes of spliced mRNAs which we designate types 1 to 5. They had a common poly(A) addition site located at coordinate 53 and all mRNAs, except the type 3 mRNAs, contained an exon located between coordinates 41 and 53. In the type 1 mRNAs this exon was connected to a very short leader sequence, located around coordinate 31. The type 2 mRNAs contained 220-400 nucleotides long leaders which were located approximately 1.5 kilobases further upstream. Two different subclasses of type 2 molecules (2A and 2B) were identified and these had slightly different leaders. The type 4 mRNAs contained a bipartite leader whereas the type 5 mRNAs carried an approximately 900 nucleotides long leader. The type 3 mRNAs consisted of a main exon located between coordinates 32 and 53, linked to the same leader as is present in the type 2A mRNAs. A cap site which presumably is utilized by the type 2A, type 3, type 4, and type 5 mRNAs was mapped at nucleotide 89 in the BPV-1 sequence. A putative cap site for the type 1 mRNAs was mapped at coordinate 31.

The 5' ends of the type 1A and type 1B mRNAs have also been studied by primar extension.

Studies have also been carried out in order to determine which of the BPV-1 mRNAs that is of critical importance for transformation. For this purpose the transforming region of BPV-1 was inserted into a retrovirus vector which has been used to transform mouse cells. The mRNAs produced in cells transformed by a mutant BPV-1 virus which contains a retrovirus LTR inserted in the Eco RI cleavage site of BPV-1 has been studied in order to further elucidate the mRNAs which have a transforming function.

Finally point mutations have been inserted by in vitro mutagenesis into critical parts of the transforming region in order to establish a correlation between the structure of different mRNAs and their transforming capabilities.

1379 EXPRESSION OF HUMAN PAPILLOMAVIRUS SEQUENCES IN CERVICAL CARCINOMA CELLS, Elisabeth Schwarz, Birgit Roggenbuck, Wolfgang Mayer, Lutz Gissmann and Harald zur Hausen, Institut für Virusforschung, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, 6900 Heidelberg, FRG.

Cell lines derived from human cervical cancer (HeLa, C4-1, 756) contain DNA sequences of human papillomavirus type 18 (HPV18, ref. 1) integrated into the cellular genome (ref. 2). HPV18 DNA is amplified in HeLa and 756 cells together with flanking cellular sequences. Almost the complete HPV18 genome seems to be present in 756 cells and is linearized due to integration by opening within a 1.15 kbp segment of the early region. In HeLa and C4-1 cells, a 2-3 kbp segment of HPV18 sequences from the E2 to L2 region is missing and one border of the integrated HPV18 DNA is also located within the 1.15 kbp region. In all three cell lines, HPV18 sequences are specifically transcribed from the E6-E7-E1 early region into poly(A)$^+$ RNAs which range in size from about 1.5 kb to 6.5 kb. The large size of some of the transcripts suggested that transciption may also include flanking cellular sequences. cDNA libraries from the poly(A)$^+$ RNAs of the three cell lines were constructed and HPV18 positive cDNAs isolated. Some of the cDNAs from 756 cells were shown to contain in addition cellular sequences which are repeated in the human genome. For further analysis of the transcribed HPV18 and cellular sequences and for prediction of putative gene products, the nucleotide sequences of the cDNAs are now determined.

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1380 IDENTIFICATION OF CAP SITE OF "EARLY" AND "LATE" COTTONTAIL RABBIT PAPILLOMA VIRUS TRANSCRIPTS AND SEQUENCES REQUIRED FOR TUMOR INDUCTION. F.O. Wettstein and M. Nasseri, Department of Microbiology and Immunology, and Molecular Biology Institute, UCLA School of Medicine, University of California, Los Angeles, California USA.

Several species of viral transcripts can be resolved by Northern blotting in virus producing and nonvirus producing tumors induced by the cottontail rabbit papilloma virus (CRPV). Two major transcripts of 1.3 and 2.0 kb are found in non-virus producing benign and malignant tumors induced in domestic rabbits. Both transcripts consist of two exons and they share a common 3' proximal exon. The 5' proximal exons are overlapping with a common 3' end. The 5' end of the 5' proximal 1.3 kb RNA exon determined by S_1 mapping is identical to the end determined by primer extension, suggesting that it represents the cap site of the RNA. Further, a TATAAA sequence is located about 30 nucleotides upstream from the cap site. The 2.0 kb RNA consists of two very similar species. The major one has a 5' end which maps approximately 70 nucleotides downstream from the minor one. Primer extension experiments result also in two bands for the 5' ends of the 2 kb RNAs identifying the same 5' ends as S_1 experiments did; indicating the presence of two different cap sites. Both sites have about 30 nucleotides upstream a TATA like sequence (TATAT, TATAA). These data suggest that the three RNA species are independently transcribed. In this context it is interesting to note that a DNA deletion confined to the "late" region and starting 0.6 kb upstream from the first cap site does not induce tumors in rabbits while a smaller deletion starting 2.1 kb upstream does induce tumors. These results suggest that sequences hundreds of base pairs upstream from the cap sites and located in the "late" region are required for expression of "early" functions necessary for tumor induction.

In virus producing cottontail rabbit papillomas three additional transcripts unique to virus producing tumors can be resolved. Two of the transcripts (4.8 and 2.6 kb) have their main bodies in the "late" region. Both transcripts consist of at least three exons. The 5' end of the 5' proximal exon of the 4.8 kb RNA identified by S_1 mapping corresponds also to the end identified by primer extension. Gradient separation of the 4.8 and 2.6 kb RNA showed that the identical exon is also present in the 2.6 kb RNA and this suggests that the 4.8 and 2.6 kb RNA are derived from the same primary transcript by differential splicing. For these transcripts, however, there is no TATA like sequence at the anticipated location.

Epithelial Cell Culture

1381 PAPILLOMAVIRUS EXPRESSION IN CULTURED KERATINOCYTES, Lorne B. Taichman, State University of New York at Stony Brook, Stony Brook, New York.

Productive replication of papillomaviruses (PVs) is observed only in keratinocytes which are in the process of terminal differentiation. We have endeavored to develop a culture model for productive HPV1 replication using human epidermal keratinocytes in culture.

When cultured newborn keratinocytes are infected with HPV1 particles there is no CPE and there are no visible intranuclear viral particles nor detectable capsid antigens. However, HPV1 DNA is present as a monomeric episome at about 100 copies per cell. HPV1 DNA replicates and persists at the same copy number for up to eight passages. Experiments have shown that viral DNA replication occurs only in the least differentiated cells in the culture and not in the cells undergoing terminal differentiation. Attempts to induce vegetative HPV1 replication by augmenting the level of keratinocyte differentiation have not been successful.

Since HPV1 replication in warts occurs in cells which are transformed, we attempted to infect lines of keratinocytes derived from naturally occurring squamous carcinomas. However, HPV1 DNA in these cells was unstable and was lost by the second to third passage.

DNA tumor viruses, with the exception of PV, do not undergo productive replication in the cells they transform. The difficulty we are encountering in developing a culture model for productive PV replication is probably related to a dual need for cellular transformation and terminal differentiation.

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1382 THE USE OF CULTURED MOUSE EPIDERMAL CELLS FOR STUDIES IN CARCINOGENESIS, Stuart H. Yuspa, Laboratory of Cellular Carcinogenesis and Tumor Promotion, National Cancer Institute, Bethesda, MD, 20205.

The ability to culture mouse epidermal cells has provided a model for delineating the mechanism of action of initiators and promoters. In vitro, a low level of extracellular calcium (<0.1 mM) selects for the basal cell population while higher levels (>0.1 mM) induce terminal differentiation. Exposure of cultured mouse keratinocytes to initiating agents results in cellular foci with altered behavior for the calcium signal. The altered cells resist the inhibitory growth signal associated with the induction of terminal differentiation by calcium (2). Similar foci are derived from cultured keratinocytes isolated from epidermis of mice initiated in vivo (3). In addition the number of foci derived in vitro is dependent on both the dose and potency of the initiating agent used either in vivo or in vitro. Thus the altered differentiation phenotype is correlated to the initiating event. Using oncogenic retroviruses as vectors, studies have been performed to assess the role of an activated ras oncogene in producing the initiated phenotype (4). An activated ras gene induces dramatic increases in epidermal basal cell proliferation, but such cells differ from initiated cells in that their growth ceases in response to the high calcium signal. However the proliferative block of sarcoma virus infected keratinocytes can be reversed by reculturing in low calcium medium. Furthermore, virus blocked cells, unlike differentiating normal cells, respond to phorbol ester tumor promoters. Promoter exposure produces enzyme changes which indicate that virus blocked cells can undergo a phenotypic reversion to cells with characteristics of a less mature cell stage. These results suggest that activation of a ras gene could result in conditionally initiated cells in which the full expression of their biological potential depends on exposure to tumor promoters.

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Transformation

1383 TRANSFORMATION BY POLYOMA VIRUS, Thomas L. Benjamin, Department of Pathology, Harvard Medical School, Boston, MA 02115, Leda Raptis, Division of Molecular Genetics, National Research Council of Canada, Ontario, Canada K1A0R9, Robert L. Garcea, Division of Pediatric Oncology, Dana Farber Cancer Institute, Boston, MA 02115

The small and middle T antigens of polyoma virus comprise dual products of the hr-t gene. In non-productive infections, the function of this gene is required for virtually all of the cellular changes accompanying cell transformation, and in productive infections it is required for efficient assembly of virus particles.[1] Oligonucleotide mutagenesis experiments have been aimed at inducing specific structural changes in these proteins. The mT protein, in the absence of small T, can elicit a full range of transformed cell behavior in established rat embryo fibroblasts. Using the dexamethasone-regulatable promoter derived from the MMTV-LTR to regulate the amount of mT being synthesized, the transformed phenotype unfolds in a definite manner depending on the level of expression of mT. Morphological changes occur with mT at a level of a few percent that of wild type viral transformants, focus formation at about 20%; growth in soft agar and tumorigenesis in syngeneic rats show proportional responses from 20 to 100% levels of mT expression. These parameters of transformation appear to depend on the amount of mT present in mT·pp60$^{c\text{-}src}$ complexes, and not the total amount of mT present in the cell. Studies of the virion assembly defect of hr-t mutants in productively infected mouse cells indicate a role of this viral "transforming" gene in phosphorylation of VP-1, while work from another lab has shown a potential requirement for VP-1 modification in the all-pentamer model of the polyoma capsid.[2]

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1384 STAGES IN THE TUMORAL PROGRESSION INDUCED IN RODENT FIBROBLASTS BY BOVINE PAPILLOMA VIRUS TYPE 1, F. Cuzin, G. Meneguzzi, B. Binétruy, G. Connan and M. Grisoni, INSERM U273 and CNRS LP7300, Centre de Biochimie, Université de Nice, 06034, Nice, France.

Four distinct stages could be described in the oncogenic transformation process induced by BPV1 in rat FR3T3 and mouse C127 cells. Stage 1 lines, selected in G418 medium after transfer of a BPV1-neo plasmid (1), maintain normal cell morphology and growth control in culture; viral DNA is present in a plasmidial form; these lines produce phenotypically transformed derivatives (Stage 2) at a low frequency, which increases after treatment with a tumour promoter; upon injection in syngeneic animals or nude mice, Stage 1 cells produce tumours after a latency period of several months, in some instances at locations distant from the injection sites. Stage 2 lines were derived either by focus selection after transfer of viral DNA or by spontaneous transformation of Stage 1 cells; although their phenotype in vitro is intermediate between that of a normal cell and that of a "typical" transformant (polyoma-, ras-transformants), they produce highly invasive tumours in the animal (2). Stage 3 cell lines were established in culture from tumours induced by Stage 2 cells (2); they maintain the viral DNA as a non-rearranged plasmid; unlike Stage 2 cells, they grow efficiently in suspension and they are even more malignant in vivo; they maintain, however, an organized cytoskeleton and remain dependent on serum factors for growth in culture. Stage 4 lines were also selected by tumour formation in the animal, but directly from Stage 1 cells; they differ from Stage 3 cells by the complete loss of actin cables and the presence of highly rearranged autonomous and/or integrated viral DNA (except one line which did not contain viral DNA any more).

The phenotypes of Stage 1 and Stage 2 cells are remarkably different from that induced, also at an early stage of transformation, by the "immortalizing" genes of other DNA viruses (polyoma plt, adenovirus E1A) or by myc oncogenes (3-5). BPV1 early genes did not in fact induce immortalization in a stringent assay on REF cells. Complementation experiments between BPV1 and the polyoma plt and pmt and cellular myc and ras oncogenes are in progress. The tumoral progression starting from these early stages and leading to the advanced malignant Stages (3 or 4 depending on the conditions of selection) may involve changes both in viral and in cellular gene expression. It is not clear whether a viral activity is still necessary in Stage 4 cells, in which apparently random rearrangements and, in one instance, a complete disappearance of the BPV1 genomes were observed.

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1336 MUTATIONAL ANALYSIS OF CELL TRANSFORMATION BY BOVINE PAPILLOMAVIRUS, Daniel DiMaio, Department of Human Genetics, Yale Univerisity School of Medicine, New Haven, CT., 06510

Site-specific mutagenesis is being used to investigate the transforming functions of BPV type 1. Using oligonucleotide-directedmutagenesis of a subgenomic fragment of BPV DNA cloned in M13mp8, a C-to-G transversion mutation was constructed at BPV nucleotide number 3081. This base change generates a UAG translation termination codon in the mRNA transcribed from open reading frame E2 in the transforming segment of BPV DNA, but it does not alter any of the other viral open reading frames. The nucleotide substitution was confirmed by restriction endonuclease cleavage and by nucleotide sequencing. An intact viral genome containing the mutation was constructed in vitro, and the biological activity of the cloned, mutant DNA was assayed in mouse C127 fibroblasts. Following calcium phosphate-mediated transfer of the full length viral genome excised from the bacterial plasmid vector, the mutant DNA wasabout 50 fold less efficient than wild type DNA in inducing the formation of dense foci of morphologically transformed cells on a monolayer of normal cells. This transformation defect is less pronounced in a different transformation assay, the ability of transfected, sparsely-plated C127 cells to form colonies containing morphologically transformed cells. The rare foci generated with the mutant contain integrated mutant DNA, and cells from these foci are able to form colonies in agarose. Genetic mapping experiments demonstrated that the constructed amber mutation is responsible for the transformation and replication defects. These results suggest that the putative protein product of BPV open reading frame E2 is involved in the processes of cellular transformation and viral DNA replication. To confirm this, we are attempting to correct these defects by the introduction of a suppressor tRNA into cells containing the mutant.

Animal Papilloma Viruses

1385 INTERACTION OF MASTOMYS NATALENSIS PAPILLOMAVIRUS GENOMES WITH CARCINOGENIC AGENTS

Eberhard Amtmann[1] and Klaus Wayss[2], [1]Institute for Virus Research and [2]Institute for Experimental Pathology, German Cancer Research Center, Heidelberg, FRG

In the Giessen strain of Mastomys natalensis skin tumors appear frequently in animals that are older than one year. Mastomys natalensis papillomavirus (MnPV) was identified as the etiologic agent of these tumors (1). In the normal skin of Mastomys extrachromosomal papilloma virus genomes were detected. The relative amount of viral DNA increased with ageing of the animals (2). The effects of agents which are active at different stages of carcinogenesis on viral DNA accumulation and tumor formation were studied. By application of the carcinogen 9, 10 dimethyl-1.2-benzanthracene mainly carcinomas and a few basaliomas were induced. These tumors contained only minute amounts of MnPV DNA. Viral mRNA transkription was detected in no case. By treatment with the tumor promoter 12-0-tetradecanoyl-phorbol-13-acetate (TPA) and the second stage promoter 12-0-retinoylphorbol-13-acetate (RPA) kerathoacanthomas and papillomas were induced. These tumors contained several thousand MnPV DNA molecules per cell. While the carcinogen DMBA had no effect on the amount of MnPV DNA in skin cells, both TPA and RPA had a strong stimulating effect.

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1386 PERSISTENCE OF SHOPE PAPILLOMAVIRUS GENOME IN SHOPE PAPILLOMA CARCINOMA COMPLEX. Yohei Ito and Kenji Sugawara, Department of Microbiology, Faulty of Medicine, Kyoto University, Kyoto 606, Japan

In 1960, papillomas were induced in domestic rabbits with DNA extracts from glycerinated cottontail rabbit papilloma tissues and from partially purified Shope papilloma virus (SPV). This was the very first case of tumor induction in mammalian hosts with subviral agent. Such experimental procedures are recently being called as transfection. Later, it was also demonstrated that transfectious DNA extracts can be obtained from tissues of all phases of the Shope papilloma carcinoma complex, domestic rabbit papillomas, primary carcinomas and transplantable Vx7 carcinomas. Transfection with DNA extracts from Vx2 carcinomas was unsuccessful despite elaborate repeated attempts. The expression of structural coat proteins of SPV was also detectable in all these variety of tissues of the system by immunofluorescence technique. This suggested, although indirectly, that the SPV genome including the late structural genes are persisting in the system.

More direct evidence was provided by detection of SPV DNA by recent technology of molecular hybridization and reassociation kinetics. The cottontail papillomas, domestic papillomas and transplantable Vx2 and Vx7 carcinomas were shown to contain 1,000-8,000, 40-400 and 10-20 viral copies per diploid cells, respectively. The efficiency of transfection also paralleled these data roughly. More recently, we have cloned SPV DNA in p322 at SalI site and inoculated such DNA preparations into skin of domestic rabbits. Intact or SalI-digested recombinant DNA induced typical papillomas at the sites of inoculation, however, the rate of positive growth (14% and 25%) was much lower than that of the DNA preparations from natural SPV (100%). SPV genomes were detectable in all tumors induced by the synthetic DNA, however, their physical state, integrated or non-integrated, seemed to differ case by case.

Our SPV studies for twenty-five years will be reviewed as summarized above and implications of these findings as a model for human neoplasia will be discussed.

1387 A COMPARISON OF THE TWO SUBGROUPS OF BOVINE PAPILLOMAVIRUSES, William Jarrett, Department of Veterinary Pathology, University of Glasgow, Bearsden, Glasgow, Scotland G61 1QH

The six currently characterised Bovine Papillomaviruses (BPV) can be divided into 2 subgroups by a range of criteria. BPV-1, 2 and 5 have the ability to transform both fibroblasts and epithelial cells in vivo and fibroblasts in vitro. The tumours they induce although appearing very different macroscopically, develop by the same 3 stages of transformation; the first 2 of these are non-permissive for viral replication. BPV-3, 4 and 6 transform only epithelial cells and thus induce true papillomas rather than fibropapillomas. The genomes of the 2 subgroups are of different lengths; sequence homology exists between members of each subgroup but not between subgroups. The second subgroup does not seem to have the so-called papillomavirus group-specific antigen. The relationships of the various viruses to naturally occurring tumours will be outlined as will implications of these models for human oncology.

1388 COMPARISON OF THE BIOLOGY OF BOVINE AND DEER PAPILLOMAVIRUSES. W.D. Lancaster and D.E. Groff. Georgetown Univ. Med. Ctr., Washington, DC 20007.

Papillomaviruses comprize a heterogeneous group of viruses which are closely related but exhibit varied activities in vivo and in vitro. The intensely studied bovine papillomavirus type 1 (BPV-1) and the less well understood deer papillomavirus (DPV) are capable of tumor formation in animals and morphological transformation of mouse cells in culture.

Early DNA hybridization studies demonstrated that BPV-1-induced tumors contained high concentrations (100-700 copies/cell) of viral sequences [1]. Naturally occurring equine connective tissue tumors as well as virally-induced equine tumors were also shown to contain BPV DNA sequences suggesting that the virus was capable of crossing the species barrier and induce tumors in an unrelated host as a result of natural infection [2]. Subsequent blot transfer hybridization experiments demonstrated a lack of integration of the BPV genome in a variety of spontaneous and experimentally-induced tumors as well as mouse cells morphologically transformed by virus [3,4]. Similar studies with DPV have revealed that the genome of this virus also does not appear to integrate while maintaining the transformed state [5].

DNA sequence analysis has revealed that the DPV genome (8371 bp) is somewhat larger than BPV-1 (7945) [6]. This size difference is reflected in a region of no open reading frames within the DPV genome separating the open reading frames of the early and late regions. The remainder of the the DPV genomic organization is similar to that of BPV-1. The degree of sequence homology (75%) exhibited between these viruses which spans both the early and late regions is remarkable. Although these two viruses have extensive DNA sequence homology, they are incapable of infecting the reciprocal host and their spectrum of biological activity shows differences. In culture, mouse cells show differential susceptibility to infection by BPV-1 and DPV which is independent of whether the cells are infected with virions or virus DNA. Whereas only BPV-1 transforms C127 cells and only DPV transforms Balb/3T3 Clone A31 cells, both viruses transform NIH/3T3 cells.

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