PAPILLOMAVIRUSES

Organizers: Peter Howley and Thomas Broker

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Poster Sessions

Squamous Epithelial Cell

I 001 REGULATION OF KERATIN GENE EXPRESSION IN DIFFERENTIATING HUMAN EPIDERMAL CELLS, Elaine Fuchs, Raphael Kopan, Robert Vassar, Marjorie Rosenberg, and Michael Henderson. Howard Hughes Medical Institute, Department of Molecular Genetics The University of Chicago, Chicago, IL 60637.

Keratins are the major differentiation-specific products of epidermis and its appendages. They can be subdivided into two groups, type I (keratins K9-K19, 40-60kd) and type II (keratins K1-K8, 53-67kd) which are both essential for assembly into 8 nm cytoskeletal filaments. In skin, keratins are expressed in a complex pattern of specific type I and type II pairs. In the basal layer of epidermis, only K5 and K14 mRNAs are expressed, but in suprabasal layers, mRNAs for K1 and K10 appear as cells commit to terminally differentiate. A third set of keratins is seen during wound-healing and in epidermal diseases of hyperproliferation. In hair follicles, basal epidermal keratins are expressed in outer root sheath cells, but hair-specific keratins are found in cortical cells. Indeed at each step of differentiation and development during skin morphogenesis, the program of keratin expression undergoes change.

Little is known about the molecular mechanisms underlying changes in keratin expression, although vitamin A seems to play a role in the process and in the general program of skin differentiation. To elucidate the mechanisms of keratin gene switching and vitamin A-mediated regulation of differentiation, we have isolated and characterized a number of human keratin genes and have made specific cRNA and antibody probes as tools for our studies. Using these tools, we have examined the temporal appearance of these keratins during early development, and have correlated changes with cell determination and morphogenesis in skin. In addition, we have coupled the use of these tools with (a) gene transfection and in vitro cell culture, and (b) transgenic mouse technology and in vivo studies, to begin to explore the regulatory pathways involved in keratin gene expression and the functional significance of the multiplicity of keratin sequences. Finally, we have used keratin expression to monitor the progression of terminal differentiation in cultured keratinocytes. These studies have led to improvements in methods to culture keratinocytes, including squamous cell carcinoma cells and HPV-16 transfected cells, such that their morphology and biochemistry in vitro is similar to that observed in vivo.

I 002 PAPILLOMAVIRUS GENE EXPRESSION ALTERS THE GROWTH AND DIFFERENTIATION OF HUMAN KERATINOCYTES.

Richard Schlegel, Laboratory of Tumor Virus Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.

Keratinocytes exhibit a characteristic pattern of cell growth and differentiation which can be modulated by the concentration of calcium and serum present in the growth medium. Under conditions of low calcium (0.15 mM or less), human keratinocytes proliferate as a cell monolayer with reduced desmosome formation. The addition of calcium and serum induces increased desmosome formation as well as cell stratification and differentiation. This program of keratinocyte differentiation is altered following transfection with specific types of papillomavirus DNA (i.e. those HPV types associated with cervical carcinoma) [Schlegel et. al., EMBO J. 7: 3181-3187, 1988]. HPV-16 and HPV-18 transfected keratinocytes are resistant to serum-induced terminal differentiation and stratify poorly. Unlike many squamous cell carcinoma cell lines, however, these HPV-transfected keratinocytes appear to express normal numbers of EGF receptors on their cell surface and to respond normally to epidermal growth factor (EGF). Also, these cells respond normally to growth inhibitory factors such as beta-transforming growth factor. Genetic analysis indicates that two HPV genes are required for this phenotype, E6 and E7. A detailed study of the biological activity of these genes in human keratinocytes is described in another abstract [Munger et. al.]. The contribution of these two genes to altered cellular proliferation and differentiation will be discussed and related to the chronology of in-vitro keratinocyte transformation.

I 003 THE REGULATION OF DIFFERENTIATION IN NORMAL AND NEOPLASTIC KERATINOCYTES. Stuart H. Yuspa, Laboratory of Cellular Carcinogenesis and Tumor Promotion, National Cancer Institute, Bethesda, MD 20892. Based on the mutagenic action required for specific chemicals to produce benign or malignant tumors, at least two genetic events occur prior to carcinoma formation in mouse skin. The first change, initiation, is commonly associated with a point mutation in a regulatory gene such as c-ras^{H} and imparts a defect in the response to normal signals for epidermal terminal differentiation. Such cells can be selected in vitro by virtue of their resistance to Ca^{2+} induced terminal differentiation. The differentiation-altered phenotype in vitro is shared by cells derived from papillomas, the pathological manifestation of the initiating event. Initiated and papilloma cells also share resistance to phorbol ester-induced terminal differentiation. In vivo, differentiation markers are aberrantly expressed in papillomas suggesting that neoplastic epidermal cells do not respond normally to maturation signals. Epidermal maturation may be initiated by the combined action of an increased intracellular Ca^{2+} and stimulation of protein kinase C activity. This signal transduction mechanism is regulated by the activation of a specific phospholipase C and the consequential stimulation of phosphatidylinositol (PI) metabolism. The enhancement of PI metabolism in response to a terminal differentiation signal is intact in some initiated cells, but these cells seem incapable of sustaining an increased intracellular Ca^{2+} signal. The importance of the defect in sustaining increased intracellular Ca^{2+} in producing the phenotype of initiated cells is supported by studies indicating that terminal differentiation can be restored in initiated cells by exposure to a calcium inonophore. Together the studies suggest that a pathway which mediates intracellular Ca^{2+} control may be a common target for initiating agents in skin carcinogenesis.

1. Roop, D. R., Krieg, T. M., Mehrel, T., Cheng, C. K. and Yuspa, S. H.: Transcriptional control of high molecular weight keratin gene expression in multistage mouse skin carcinogenesis. Cancer Res. 48: 3245-3252, 1988.
2. Jaken, S. and Yuspa, S. H.: Early signals for keratinocyte differentiation: Role of Ca^{2+}-mediated inositol lipid metabolism in normal and neoplastic epidermal cells. Carcinogenesis 9:1033-1038, 1988.

Discussion: HPV Pathogenesis

I 004 **ANALYSIS OF REPEATED CERVICAL SWABS FROM PAP-NEGATIVE WOMEN FOR THE PRESENCE OF HPV 16 DNA** Lutz Gissmann, Tatjana Kirchhoff, Christina von Knebel Doeberitz, Ingrid Jochmus-Kudielka, Gabriele Meinhardt[1], Achim Schneider[1] Deutsches Krebsforschungszentrum, Heidelberg Fed. Rep. Germ. [1]Universitäts-Frauenklinik, Ulm Fed. Rep. Germ.

As suggested by earlier studies the frequency of clinically inapparent infections with HPV 16 at the uterine cervix seems to be higher than determined by the analysis of a single swab. We therefore collected 10-12 subsequent swabs (at six weeks' intervals) from 20 sexually active women presenting without any clinical or cytological indication for a papillomavirus infection. By Southern blot hybridization five of them were demonstrated to harbor HPV DNA (two HPV 16, two HPV 18, one HPV X) in only one of their swabs.

A total of 168 swabs of the 15 HPV negative women were tested by the poly chain reaction for the presence of HPV 16 DNA. Using an oligonucleotide from the amplified region for hybridization a specific product was demonstrated in 6o.1% of the swabs. In total, 13 out of 15 women analysed contained HPV 16 DNA in 33-100% of their swabs taken during the course of the study. In contrast, testing of a single swab (taken at any time) of those 15 women would have resulted in only 4-8 positive individuals.

Host Factors in Viral Gene Expression

I 005 CELLULAR TRANSCRIPTIONAL CONTROL MECHANISMS AS DEFINED WITH ADENOVIRUS, Joseph R. Nevins, Department of Microbiology-Immunology, Duke University Medical Center, Durham, North Carolina 27710

Previous experiments have identified a cellular factor termed E2F that appears to be involved in the transcriptional induction of the adenovirus E2 gene. The E2F factor binds to promoter sequences critical for transcription, the level of active factor increases at least 30-fold in virus-infected cells, and the purified factor stimulates transcription in vitro dependent on E2F binding sites. Furthermore, the activation of E2F binding activity is a post-translational event not requiring new protein synthesis. We have now developed an assay for the in vitro activation of E2F binding activity, using cell-free extracts. Extracts of uninfected HeLa cells exhibit a very low level of E2F activity, but upon incubation with a fractionated extract from adenovirus infected cells, there is an ATP-dependent increase in the level of E2F. This increase does not occur using an equivalent fractionated extract prepared from uninfected cells or E1A mutant infected cells. Treatment of active E2F factor with phosphatase inactivates the E2F binding activity and incubation of the phosphatase-treated factor with the infected cell extract restores E2F activity. Finally, phosphatase-inactivated E2F can be activated by incubation with purified cAMP protein kinase. We conclude that the phosphorylation of an inactive E2F, activates the ability of the factor to bind to DNA and stimulate transcription.

In addition to the E2F factor, our previous experiments have identified an inducible cellular factor termed E4F, that binds to sequences in the adenovirus E4 promoter which have been shown to be critical for transcription and induction. We have purified the E4F factor to apparent homogeneity, identified the active factor as a single 50 kD polypeptide distinct from the 54 kD E2F factor, and shown that it is capable of stimulating E4 transcription in vitro. The activation of E4F also appears to involve a phosphorylation event since treatment of E4F with alkaline phosphatase abolishes activity, and incubation of the phosphatase-inactivated factor with an extract from virus infected cells restores activity. All of these properties are analogous to those of E2F and we therefore suggest that the coordinate control of E2 and E4 transcription may involve a common activation of multiple factors through a phosphorylation event.

I 006 AN INTERPLAY OF CELLULAR AND VIRAL FACTORS REGULATE HPV18 TRANSCRIPTION, Moshe Yaniv, Alejandro Garcia Carranca*, Nathalie Dostatni, Rosemary Sousa, Isabelle Giri, Bruno Bernard°, Brigitte Bourachot and Françoise Thierry°°, Unité des Virus Oncogènes, Institut Pasteur, Paris, France, *CINVESTAV-IPN, Mexico, °CIRD, Sophia Antipolis, Valbonne, France, °° Laboratory of tumor virus biology, NIH, Bethesda, USA.

Dissection of the long control region (LCR) of HPV18 revealed that it contains a tissue and species specific promoter as well as a constitutive and an E2-inducible enhancer. The constitutive enhancer contains binding sites for AP1/jun transcription factor, NF1/CTF and an epithelial cell specific factor[1]. Contrary to the situation observed with enhancer constructions, the intact HPV18 control region is strongly repressed by the full length BPV1 E2 in Hela or SW13 cells[2] and by the homologous E2 in human keratinocytes (B. Bernard, C. Bailly, M. Darmon, F. Thierry and M. Yaniv, submitted).

The viral E2 protein binds as a dimer to each palindromic recognition site[3,4].The mechanism of transactivation by this protein was studied with tkCAT vectors containing multiple E2 binding sites 5', 3', or both, relative to the transcription unit. A clear synergy of activation is seen between binding sites present in both extremities of the gene. Whereas in most cell lines like Hela or CV1 a minimum of two sites is required for activation, a single site gives 4 to 17 fold activation in SW13 or F9 cells, respectively.

A hybrid gene containing the c-jun DNA binding domain and the N-terminal part of E2 strongly activates transcription of promoters containing the jun binding site. These experiments confirm that the N-terminal region of E2 has all the properties of a transactivating domain[4-6].

1. A. Garcia Carranca et al. 1988. J. Virol. 62, 4321-4330. 2. F. Thierry and M. Yaniv. 1987. EMBO J. 6, 3391-3397.3. N. Dostatni et al. 1988. EMBO J. 7, 3807-3816.4. A. McBride et al. 1988 Proc. Natl. Acad. Sci. USA, in press.5. I. Giri and M. Yaniv. 1988. EMBO J. 7, 2823-2829.6. P. Lambert et al. 1989 Genes and Dev., in press.

The Papillomavirus Transcriptional Program

I 007 **REGULATION OF BOVINE PAPILLOMAVIRUS TYPE 1 LATE GENE EXPRESSION,** Carl C. Baker, Laboratory of Tumor Virus Biology, National Cancer Institute, Bethesda, MD, 20892

The late genes of bovine papillomavirus type 1 (BPV-1) are expressed only in the differentiated keratinocytes of the fibropapilloma and are not expressed in either the fibromatous portion of the fibropapilloma or in BPV-1 transformed C127 cells. Transcription of the BPV-1 genome in productively infected tissues differs from transcription in nonproductively infected tissues and cells by the use of a wart specific promoter and polyadenylation site (EMBO J. 6:1027-1035, 1987). Further studies indicate that multiple negative regulatory elements within the BPV-1 late region block late gene expression in BPV-1 transformed cells in culture. Nuclear run-off analysis of BPV-1 transformed C127 cells indicates that transcription attenuation or termination within the late region is partially responsible for the block of late expression.

A series of chloramphenicol acetyltransferase (CAT) eukaryotic expression vectors have been designed for the analysis of polyadenylation site usage, transcription termination, and mRNA destabilization sequences and were used to study the regulation of BPV-1 late gene expression. A fragment containing the BPV-1 late polyadenylation signal stimulates CAT expression from a CAT vector lacking a polyadenylation signal as efficiently as a fragment containing the BPV-1 early polyadenylation signal when assayed by transient expression in BPV-1 transformed C127 cells. In addition, nuclease S1 analysis was used to confirm that the late polyadenylation site is used in transient expression assays and furthermore that this polyadenylation site is also used at low levels in cycloheximide treated ID13 cells. This suggests that late gene expression is not controlled at the level of polyadenylation. Analysis of BPV-1 fragments containing sequences upstream of the BPV-1 late polyadenylation signal suggested the presence of a negative regulatory element in this region, however. A 117 bp fragment (n. 7029-7145) consisting of the extreme 3' part of the L1 ORF and the 3' untranslated region (UTR) inhibits CAT expression approximately 20 fold when cloned into the 3'UTR of a CAT vector. Quantitative nuclease S1 analysis indicates that this inhibition correlates with a comparable decrease in the steady state levels of CAT RNA. This element has no effect when cloned into an intron upstream of the CAT gene or downstream of the polyadenylation site, indicating that it must be present in the mRNA to inhibit expression. The most likely mechanism of inhibition is mRNA destabilization; however, experimental confirmation of this hypothesis has not been possible due to the low steady state levels of CAT mRNA. Transient expression assays are also being used to study termination of transcription within the late region. Specifically, an approximately 450 bp fragment from the 5' late region inhibits CAT expression greater than 100 fold when cloned into an intron upstream of the CAT gene, as would be expected for a strong transcription terminator. Experiments are currently in progress to confirm that this inhibition is due to transcription termination and to further map the transcription terminator.

I 008 **TRANSCRIPTIONAL TRANSACTIVATION BY THE BPV-1 E2 GENE IN YEAST**

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The papillomavirus E2 transcriptional transactivator is representative of a class of transcriptional modulators which activate transcription through direct binding to *cis*-acting DNA sequences. In this study we measured the capacity for the BPV-1 E2 transactivator to function in *Saccharomyces cerevisiae*. The full-length E2 ORF was placed under transcriptional control of the regulatable, yeast PHO5 promoter. Under PHO5 induced conditions, a 48 kd polypeptide was immunoprecipitated using E2 specific antisera, identical in size to the full-length E2 gene product present in BPV-1 infected mammalian cells. Likewise, specific binding to the E2 DNA binding site, 5'-ACCN$_6$GGT-3', was detected by the gel retardation assay. Expression of the bovine papillomavirus E2 transactivator led to the stimulation of transcription from a yeast promoter having E2 DNA binding sites present *in cis*. Whereas a single E2 DNA binding site was sufficient for transactivation, a strong cooperative effect was observed with two E2 DNA binding sites. The level of transactivation was dependent upon the position of the E2 DNA binding sites in relation to the yeast promoter, with the maximal effect demonstrated when the binding sites were positioned upstream and a partial effect when the binding sites were located in a downstream intron. Deleted E2 proteins, lacking part of the transactivation or DNA binding domains failed to activate transcription in yeast, similar to their behaviour in mammalian cells. Replacement of the N-terminal region of the E2 transactivation domain with a synthetic amphipathic helix partially restored the transactivation function, however, it did not result in a molecule which exhibited cooperativity between neighboring E2 DNA binding sites. The HPV-16 E2 gene product was also found to transactivate in yeast. These data indicate that mammalian viral transcriptional factors can function in a lower eukaryotic host cell, confirming the strong conservation of transcriptional machinery among diverse organisms.

I 009 ANALYSIS OF THE BPV E2 PROTEINS. John T. Schiller[1], Elliot J.Androphy[2], Thomas H. Haugan[3], Nancy L. Hubbert[1], Douglas R. Lowy[1], and Lubomir P. Turek[3]. [1]National Cancer Institute, Bethesda, MD, [2]New England Medical Center, Boston, MA, [3]U. Iowa, Iowa City, IA.

Products of the E2 ORF regulate the transcription of the viral genome in BPV transformed cells. In these cells, we have identified three E2 encoded proteins of 48, 31 and 28 kd, with a common C-terminus. Four functions have been ascribed to the BPV ORF: specific trans-activation that is dependent upon $ACCN_6GGT$ motifs in cis, general trans-activation that does not depend on specific sequences in cis, specific trans-repression that is dependent on the same motif, and motif specific DNA binding. To understand the mechanism of E2 mediated transcription regulation, we have generated a series of E2 mutants and used them to determine the functional domains contained in each of the three E2 proteins. The minimum domain required for specific binding to the motif was mapped to the shared C-terminus, indicating that all three function as DNA binding proteins. The N-terminal third of the protein, which is only included in the full-length 48 kd protein, was necessary and sufficient for low level general trans-activation of a variety of heterologous promoters. High activity motif specific trans-activation required both an intact N- and C-terminus but sequences in the middle of the ORF were dispensable. These results indicate that the full-length protein is the trans-activator and consists of an N-terminal activating domain that probably interacts with conserved elements of the basic transcription initiation complex linked to a C-terminal DNA binding domain that determines promoter specificity. The domain required for repression of E2 mediated trans-activation co-mapped with the DNA binding domain, suggesting that repression is due to competitive inhibition of E2 trans-activator motif binding. Since both the predominant 31 kd species and the 28 kd E8-E2 spliced product contain this domain but not the activation domain, we conclude that they both function as inhibitors of the E2 trans-activator.

Discussion: Transcriptional Regulation

I 010 DUPLICATION OF CONTROL SEQUENCES IN HPV 6 FROM ATYPICALLY LOCALIZED CONDYLOMATA ACUMINATA, Reinhard F. Kulke[1], Gerd E. Gross[2], and Herbert Pfister[1], Institut für klinische und molekulare Virologie, Friedrich-Alexander-Universität, 8520 Erlangen[1], Dermatologische Klinik, Universitäts-Krankenhaus Eppendorf, 2000 Hamburg[2], FRG.

Human papillomavirus (HPV) 6 usually induces tumors of the genital, oral or laryngeal mucosa. An HPV 6-related DNA with 8.3 kb was detected in an extrachromosomal state in atypically located warts of the mamilla and was molecularly cloned. The identity of the cloned HPV DNA with the viral DNA in the biopsy was confirmed by comparative restriction enzyme cleavage analysis, which showed typical HPV 6 DNA fragment patterns except for a 0.2 kb larger PstI B fragment. Sequencing revealed an exact 235 bp duplication encompassing nucleotides 7681 to 7896. This area is just upstream from the putative early promotor and completely contained within the 459 bp repeat described by Boshart and zur Hausen (J. Virol. 58, 1986, 963-966) in an isolate from a Buschke-Loewenstein giant condyloma. In the 235 bp repeat there is a 20 bp insertion, part of which forms an element described by Rando et al. (Virology 155, 545-556, 1986) to enhance transcriptional activity. The genetic changes observed with our isolate may influence early gene expression and tissue tropism.

Transformation: Animal Papillomaviruses

I 011 COOPERATIVE INTERACTIONS INVOLVING THE E2 TRANSACTIVATOR, THE E5 AND THE E6 PROTEINS OF BOVINE PAPILLOMAVIRUS TYPE 1 AT SUCCESSIVE STAGES OF THE TRANSFORMATION PATHWAY OF RAT FIBROBLASTS (IMMORTALIZATION, NON-ONCOGENIC TRANSFORMATION, TUMORAL PROGRESSION)

François Cuzin, Unité INSERM 273, Université de Nice, 06034, Nice, France.

Successive stages of the tumoral pathway induced by BPV1 in rat fibroblasts include (i) immortalization of primary rat embryo fibroblasts (REF) cells, efficiently induced by wild type BPV1 (as well as by HPV16), (ii) establishment of autonomously replicating genomes in either primary cells or cells of immortalized lines without immediate transformation (previously designated Stage 1)[1], (iii) progression of these cells to phenotypically transformed and tumorigenic states (Stages 2-4). Immortalization is a two-stage process, in which the permanent establishment in culture is dependent on the E6 function, but which occurs only after an initial mitogenic stimulation is provided either by the E2 transactivator or by the E5 protein. Establishment of autonomously replicating genomes requires the E1 and E2 proteins, but not E5, E6 or E7. At this stage, expression of oncogenic transformation depends at this stage on the establishment of a high rate of transcription of the viral genome. This transcriptional activation requires both the E2 and E5 gene functions. In addition to its previously recognized direct role in the expression of transformed growth properties, E5 expression thus appears to result in changes in viral, and presumably in cellular transcriptional controls. On the other hand, the role of the E2 transactivator at the initial stage of immortalization indicates that, in addition to its previously recognized effects on the regulation of BPV1 genes, it exerts direct effects on cell physiology, presumably by transactivation of cellular promoters. No hypothesis can be formulated yet on the molecular basis of the function of the third viral oncogene, E6.

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[1] Binétruy et al., 1987, Cancer Cells, Cold Spring Harbor Laboratory, 5, 223-227

I 012 THE E5 TRANSFORMING GENE OF BOVINE PAPILLOMAVIRUS. Daniel DiMaio, Jeffrey Settleman, Bruce H. Horwitz, and Amin Fazeli. Department of Human Genetics, Yale University School of Medicine, New Haven, CT.

The E5 gene of bovine papillomavirus encodes a 44 amino acid membrane-associated protein capable of inducing stable transformation of established lines of rodent cells. To examine the acute effects of the E5 protein, we have constructed a BPV/SV40 recombinant virus that efficiently expresses the E5 gene in infected cells. Acute expression of the E5 protein stimulates DNA synthesis in quiescent mouse C127 cells and induces proliferation of these cells and dramatic changes in their morphology. The extent of these effects parallels closely the dose of E5 gene, suggesting that acute transformation is not an all-or-nothing phenomenon in this system. Moreover, the rapid occurance of these effects in the great majority of cells expressing the E5 protein indicates that E5-mediated acute transformation does not require rare cellular events. To initiate a structure/ function analysis of the E5 protein and to determine the relationship between acute and stable transformation, we have determined the activity of numerous E5 mutants in both the acute transformation assay and a focus forming assay in C127 cells. These experiments indicate that an uninterrupted stretch of hydrophobic amino acids in the middle of the protein is essential for efficient acute and stable transformation. Many variant hydrophobic amino acid sequences are compatible with efficient transformation, a result consistant with the suggestion that the hydrophobic portion of the E5 protein ensures correct membrane localization. At the carboxyl-terminal amino acids that are conserved in the E5 proteins of related fibropapillomaviruses, non-conservative amino acid substitutions result in severe defects in acute and stable tranformation, indicating that the carboxyl-terminal portion of the protein plays a crucial role in these biological activities. The good correlation between the activities of the mutants in the acute and the stable transformation assays suggests that the acute responses we monitor following E5 expression may well reflect the activities of the protein essential to initiate stable transformation.

I 013 EPSTEIN-BARR VIRAL LATENCY DISRUPTION BY THE ZEBRA GENE PRODUCT, G. Miller, N. Taylor, J. Countryman, C. Rooney, D. Katz, J. Kolman, E. Grogan, L. Gradoville, Yale University School of Medicine, New Haven, CT 06510. Experiments will be summarized which address three general questions about the EBV BZLF1 gene which disrupts latency. The first is "what factors regulate expression of the gene?" The second is "which other EB viral genes are activated as the result of ZEBRA expression?" The third is "what is the biochemical mechanism of ZEBRA action?"

At least three variables have been identified which affect ZEBRA expression; these are genome rearrangements such as occur in defective virus, cell background, and the response to different inducing agents. In the standard EBV genome ZEBRA is repressed; in certain defective genomes ZEBRA is constitutively expressed. EBV converted BL cells tightly regulate ZEBRA expression, whereas more permissive cells, such as EBV immortalized marmoset cell lines permit ZEBRA expression. The inducing agents TPA and butyrate seem to act by releasing transcription of the BZLF1 gene, but their effects vary with genome configuration and cell background.

Cell lines have been created which stably express ZEBRA from BZLF1 maintained on oriP plasmids. While some of these cell lines express EBV late genes and produce virions, others express only a group of early genes. Among the early genes activated is BZLF1 itself, suggesting an autostimulatory loop. Other activated early genes include products recognized by Pearson's R3, 5B11 and K8 monoclonal antibodies. Whether ZEBRA activates expression of these three genes directly or through a cascade remains unknown.

Preliminary experiments indicate that ZEBRA is a DNA binding protein and that the DNA binding domain is located in the carboxy half of the protein.

Virus-Host Cell Interaction

I 014 CHARACTERIZATION OF THE HERPES SIMPLEX VIRUS PROTEINS INVOLVED IN DNA REPLICATION, Mark D. Challberg and Paul D. Olivo, Laboratory of Viral Diseases,National Institute of Allergy and Infectious Diseases, Bethesda, MD 20841

Using a transient complementation assay, we have shown that seven Herpes SimplexVirus (HSV) genes are both necessary and sufficient to support the replication viral DNA in infected cells. Two of these genes (pol and dbp) encode well-known DNA replication proteins (the DNA polymerase and the major single-stranded DNA binding protein, ICP8), Using the predicted amino acid sequences derived from DNA sequence analysis, we have raised rabbit antisera against the product of all seven genes, and have used these reagents to identify these proteins in infected cells. All seven proteins localize to the nucleus and are expressed in a manner consistent with the idea that they are the products of early genes. Various immunological assays suggest that four of these proteins (UL5, UL8, UL9 and UL52) are made in infected cells in very low abudance relative to the other three. In order to improve our ability to study these proteins, we have expressed UL5, UL8, UL9 and UL52 in insect cells using the baculovirus expression system. The HSV proteins made in insect cells are immunoprecipitable with the appropriate antisera and the size of each protein is indistinguishable from the corresponding protein made in HSV-infected cells.

Using these reagents, we have shown by an immunoassay for protein DNA interaction that the UL9 protein binds specifically the the HSV origins of replication. DNase I footprint analysis has shown that the UL9 protein interacts with two related sites within the origin, located on each arm of a nearly perfect palindrome.

Recombinant UL9 protein has been purified to homogeneity. The sedimentation coefficient of the purified protein in glycerol gradients (~7.5 S) suggests that UL9 exists predominately as a homodimeric structure. The interaction of purified UL9 with wild-type and mutated versions of the origin has been studied in some detail. Our results suggest that: 1) the binding of UL9 to site II (the right arm of the ori palindrome) correlates well with the ability of the origin to function in vivo, and 2) there is a cooperative interaction between UL9 bound at both arms of the origin palindrome

I 015 REPLICATION OF BOVINE PAPILLOMA VIRUS TYPE 1 ; IN VIVO AND IN VITRO STUDIES, Monika Lusky[1,2], James B. Lin[1], Philippe Clertant[3], Sabine Santucci[3], [1]Zentrum fuer Molekulare Biologie, Universitaet Heidelberg (Germany), [2]Dept. of Microbiology Cornell Medical College, New York, NY 10021 (USA), [3](Inserm-U273, Centre de Biochimie, Campus Valrose, 06034 Nice (France).

Genetic analysis has revealed that the BPV genome encodes at least six different genes whose products are directly or indirectly involved in the establishment and the stable maintenance of the viral genome as a stable nuclear plasmid in mouse C127 cells. The E1-R gene product, encoded in part by the central and 3' portion of the E1 ORF is absolutely required for early transient replication, perhaps as an initiator protein. This notion is supported by the observation that the sequence of this putative protein displays structural features in common with the ATPase and helicase domain of a viral initiator protein, the simian virus 40 (SV40) large-T antigen (Clertant and Seif, 1984 Nature 311). To further substantiate the role of E1-R in viral plasmid replication we have generated a new set of mutants by in frame insertion of two and three amino acids throughout the E1 ORF. Transfection experiments and temperature shift assays show that the stable plasmid replication of two such mutants is temperature sensitive. These results and complementation studies indicate a direct requirement of the E1-R gene product in stable plasmid replication.

Several BPV encoded proteins have been detected immunologically, including a phosphoprotein encoded by the BPV E1-M region (Thorner et al. 1988, J. Virol. 62). However, serological techniques have failed to detect any E1-R product. Furthermore, the molecular structure of the E1-R gene is not known. To gain some insight into the putative biochemical functions of E1-R we have introduced the whole E1 ORF as well as its C-terminal 70% portion into a heterologous expression system. Several distinct E1-R proteins were expressed in Vaccinia virus vectors. Subcellular localization studies and indirect immunofluorescence have revealed a nuclear localization of the Vaccinia expressed E1 products. The proteins were purified by conventional and immunoaffinity chromatography. The properties of these E1 proteins in an *in vitro* replication system and their biochemical activities will be discussed.

I 016 INHIBITION OF CELLULAR GENE EXPRESSION IN ADENOVIRUS-TRANSFORMED CELLS, Alex J. van der Eb, Rienk Offringa, Marc Timmers, Hans van Dam, Ingeborg Meijer, Hans Bos and Alt Zantema. Laboratory of Molecular Carcinogenesis, Sylvius Laboratories, P.O.Box 9503, 2300 RA Leiden, The Netherlands.

Cells transformed by human adenoviruses have been shown to be relatively independent of growth factors for their proliferation (Timmers et al., 1988). Since there is no clear evidence that this property can be explained by the autocrine production of growth factors by the transformed cells, an alternative explanation would be that the growth factor independence is caused by the ability of the viral transforming genes to substitute for growth factor responses. Since region E1A is known to possess both transcription stimulating and transcription suppressing activity, we have investigated the effects of adenovirus transformation on the expression of a number of growth factor-inducible genes, including the c-myc, the JE, the collagenase and the stromelysin gene. It was found that c-*myc* expression is reduced in Ad5-transformed and in most Ad12-transformed cells. The PDGF-inducible JE gene is also strongly suppressed both in Ad5- and Ad12-transformed cells. The repressing effect is a function of the E1A region, more specifically of conserved region 1 (one of the two conserved regions in E1A essential for transformation and enhancer repression). The genes coding for the excreted proteases collagenase I and stromelysin are similarly repressed by Ad5 and Ad12 E1A. Repression of JE, collagenase and stromelysin was found to occur at the level of transcription initiation, indicating that the inhibitory activity is directed towards the promoter of the genes. Transient expression assays with CAT constructs driven by the collagenase I promoter are being used to identify the sequences in the promoter that are involved in the repression. The observation that c-*fos* expression is still inducible to normal levels by serum indicates that the growth factor signal transduction pathway (or at least the part that is responsible for c-*fos* induction) is unaffected by E1A. Together, the results suggest that E1A inhibits the expression of a number of serum-inducible genes by actively suppressing their promoters. In the case of the c-*myc* gene, this suppression does not appear to be a prerequisite for cell transformation since in some Ad12-transformed cells c-*myc* expression is almost normal. If it is assumed that c-*myc* has an essential role in the regulation of cell proliferation our data imply that E1A is capable of taking over its function. Finally it was found that oncogenicity in immunocompetent animals of Ad-transformed baby rat kidney cells is strictly correlated with the suppression of the 27kD heat shock gene.

Discussion: Human Papillomaviruses: Immunology, Diagnosis and Therapy

I 017 GENITAL HPV INFECTIONS: DIAGNOSTIC PROBLEMS AND THERAPEUTIC CONSIDERATIONS

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Because of the lack of feasible tissue culture systems and antibody determination techniques, methods based on morphological approaches play a central role in HPV diagnosis, i.e. macromorphology, colposcopy, cytology and histopathology. These are supplemented by immunohistochemical (also based on light microscopy) and DNA-hybridization techniques (Southern blot, dot blot, FISH, sandwich, and in situ). Because of the fact that these morphological techniques are extremely difficult to standardize (e.g., morphometry, image analysis), they are subject to major variations, both interobserver and intraobserver (as shown by kappa statistic). Examples of such sources of variation (leading to discrepant results in HPV diagnosis among different groups of workers) include among others: a) the divergent colposcopic nomenclature, b) lack of unanimous cytological criteria, c) divergent views about the CIN concept, d) different reagents and varying protocols in tissue processing for immunohistochemistry, and e) lack of standardized hybridization techniques. Based on our experience on prospective follow-up of over 1.000 patients (950 women and 250 men) since 1981, the reliability of HPV diagnosis can be improved by combining the different diagnostic techniques, most importantly, colposcopy, cytology, histology and different DNA-hybridization methods. Despite the optimal diagnostic set-up, discrepancies still frequently arise in unequivocal confirmation of HPV involvement especially in the male genitalia, in the female external genitalia as well as in the outer one third of the vagina. Unfortunately, the development of therapeutic measures applicable to eradicate genital HPV infections has not followed the rapid progress in understanding the basic biology and the clinical significance of these infections. The backbone of the treatment is still based on traditional surgical techniques (conization and cryosurgery), recently supplemented by laser voporization. The cure rate of cervical and vaginal HPV infections after laser vaporization was shown to be practically identical to that obtained by cryotherapy, being significantly higher than the spontaneous regression rate, suggesting that treatment by either cryotherapy or CO_2-laser vaporization significantly changes the natural history of genital HPV infections. In controlled clinical trials, interferons objectively regress the genital warts, but a number of key unanswered questions still exists. In the future, perhaps combined therapies, such as laser surgery in combination with interferons or other chemotherapeutics might prove the most effective means to treat these patients.

Transformation: Human Papillomaviruses

I 018 PAPILLOMAVIRUS PROTEINS AND TRANSFORMATION.

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Detailed analyses of the genomes of BPV-1 and HPV 16 have shown that transforming activity is related to certain specific open reading frames (ORFs), defining them as the transforming genes of the corresponding virus. However these are different in the two cases, E5 and E6 in BPV-1 and E7 in HPV 16 and possible reasons for this difference will be discussed. Other ORFs may contribute additional elements of the transformed phenotype, both directly and indirectly via their effect on the expression of the transforming gene(s). With HPV 16 the transforming activity of the E7 gene is affected not only by the presence of other ORFs but also on the promoter construct used. An activated oncogene is normally required for efficient transformation of primary epithelial cells, although HPV 16 alone can extend the life span of keratinocytes. The spectrum of oncogenes active here is critically dependent on whether expression of the E7 gene is driven by homologous or heterologous promoters. Similarly the response to steroid hormones and tumour promoters depends on the presence or absence of elements from the viral regulatory region.

In addition to their contribution to in vitro transformation, genes from the viral early region may contribute to both the efficiency of tumour production and the type of tumour produced.

I 019 HOST FACTORS REGULATING CONDYLOMATOUS TRANSFORMATION BY HPV-11, John W. Kreider, Richard Zaino, Patricia A. Welsh and Susan D. Patrick, Departments of Pathology, and Microbiology and Immunology, The Milton S. Hershey Medical Center, Hershey, PA 17033. We have developed an experimental system which allows the condylomatous transformation of human tissues (Nature 317:639, 1985). In this system, 1-2 mm fragments of human tissues are infected with HPV-11 virions and transplanted beneath the renal capsule of athymic mice. After three months, condylomas are produced with high frequency and they grow vigorously. However, athymic mice may not provide an optimal environment for the growth of normal and papillomavirus-transformed human keratinocytes. This possibility is supported by several observations: 1)Occasional condylomas grow only temporarily. Histological examination demonstrates a core of keratinized cells, often surrounded by a dense mononuclear leucocytic infiltrate, and a complete absence of surviving condylomatous epithelium. 2)Orthotopic xenografts of human skin progressively contract and are lost. 3)We and others have found that cultured human keratinocytes transformed in vitro with HPV plasmids do not form tumors. These observations suggest that athymic mice may destroy human papillomavirus-infected cells via a nonspecific response probably mediated by natural killer cells and/or macrophages.

The objective of the present study was to improve the survival and growth of normal and papillomavirus-infected human tissues in athymic mice by administration of agents which are known to decrease natural killer cell and/or macrophage activity. These agents include beta-estradiol, antibody to asialo GM1 determinant of natural killer cells, cyclophosphamide, splenectomy, silica, and cyclosporine A. We examined the effect of the agents on experimental condylomas, orthotopic human foreskin grafts, and the SIHA cervical cancer cell line which carries HPV-16. We found that treatment with beta-estradiol significantly increased the growth rate and HPV-11 DNA and mRNA content of experimental condylomas. Beta-estradiol also eliminated the contracture of human foreskin orthografts. The growth rate of SIHA cervical cancer cell line was also increased by beta estradiol. Treatment with antibody to the asialo-GM1 determinant of natural killer cells improved the growth rate of SIHA cells, but had no effect on skin graft survival. Cyclophosphamide treatment prior to grafting did not alter skin graft survival, but did improve the growth of SIHA cells. Splenectomy improved skin graft survival, but had inconsistent effects on growth of SIHA cells. Cyclosporine A and silica were ineffective in all systems.

We conclude that beta-estradiol produced the strongest and most consistent benefits, promoting the growth of human normal skin, condylomas, and cervical carcinoma cells. Beta-estradiol probably has multiple effects in this system, including direct hormonal stimulation of the human cells as well as lymphoreticular depression. Inconsistent effects of the other agents suggest that they are less reliable and that mechanisms of destruction are probably not the same for the various types of human tissue grafts.

Pathogenesis, Diagnosis, Transcription, and Epidemiology

I 100 DETECTION OF HUMAN PAPILLOMA VIRUS IN LUNG OF SYRIAN HAMSTER EXPOSED TO LASER SMOKE, Heidi J. Agostini and David J. Giron, Department of Microbiology and Immunology, Wright State University, Dayton, OH 45435. Warts caused by Human Papilloma Viruses (HPV) are commonly removed using laser surgery. This procedure creates smoke which may contain viable HPV and thus is potentially dangerous to those in attendance. A study was initiated to determine if infectious HPV is present in laser smoke. Syrian hamsters were exposed for two years to laser smoke emitted during cauterization of wart tissue in human patients. The lungs of the animals were removed and embedded in paraffin. In situ hybridization was performed using biotin or [35S]-labelled HPV 6,11,16 and 18 cDNA probes. Preliminary results showed that HPV 18 was present in the lung of 1 out of 10 experimental animals. In addition to the presence of HPV 18, gross leukocyte infiltration was also noted. These data suggest that viable HPV is present in laser smoke and that the syrian hamster might be permissive for the replication of the HPV genome. Studies are currently underway to confirm these observations.

I 101 A KERATINOCYTESPECIFIC FACTOR BINDS TO AN ENHANCER IN HPV-16.

A. Alderborn[1], T.P. Cripe[2], P. Bergman[1], T.H. Haugen[2], L.P. Turek[2] and U. Pettersson[1].

[1]Department of Medical Genetics, Biomedical Center, Box 589, S-751 23 Uppsala, Sweden. [2]VAMC and University of Iowa, Iowa city, IA 52242.

The E6-E7 region of human papillomavirus type 16 is actively transcribed in HPV-positive carcinomas. The HPV-16 regulatory region (URR) upstream of the P97 promoter contains sequences necessary for the activation of this promoter. About 300 bp upstream of P97, a minimal keratinocyte-dependent enhancer has been identified. This region is activated by cellular factors in cervical carcinoma cells as well as in uninfected human keratinocytes (Cripe et. al. (1987) EMBO J. 6, 3745-3753). By DNase I protection analysis we have identified the DNA-protein interactions in this region. Nuclear factors from HeLa and HaCaT cells were shown to protect three distinct regions. The first region contains an octamer with a high degree of homology to the consensus cytokeratin (CK) sequence AAPuCCAAA. This octamer is found upstream of several cytokeratin genes as well as the human involucrin gene (Blessing et. al. (1987) EMBO J. 6, 567-575). The second region contains a CAAT binding protein/nuclear factor 1 (CBP/NF-1) consensus motif. The third protected region contains an activator protein 1 (AP-1) binding site. Genetic analysis of this enhancer region shows that two of these sequences, the CK and the NF-1 motifs, are necessary for full enhancer activity, whereas the AP-1 motif can be deleted without inactivating the CK enhancer (Cripe et. al., this workshop). Footprint analysis using nuclear extracts from human B-lymphocytes shows a different protection pattern.

I 102 CELLULAR PROTEINS BINDING TO THE 5' END OF THE UPSTREAM REGULATORY REGION OF HPV-11, Karen J. Auborn, Department of Otolaryngology, Long Island Jewish Medical Center, New Hyde Park, NY 11042. We previously determined that the 5' end of the URR of HPV-11 has specific DNA-protein binding using cell extracts. These presumptive cis regulatory elements span nt 7429-7496 and represent at least 3 distinct binding sites. A cellular protein, cEBP known to interact with SV40, murine sarcoma and polyoma viral enhancers binds sequences identical to one of the binding sites. The cloned gene to cEBP in lambda GT11 (courtesy of S. McKnight, Carnegie Institute) was expressed and tested for specific binding to the HPV-11 5' fragment. The cEBP strongly bound to the fragment but not herring sperm DNA or pBR322 DNA. Synthetic oligomers to the binding motifs are being tested for specific binding to protein extracts using Southwestern blotting. Preliminary results indicate that there are differences in binding proteins (presumptive trans regulatory elements) from HeLa nuclei and laryngeal papillomas as well as differences in binding to the different motifs. This work was supported by grants from Long Island Jewish Medical Center and The Council for Tobacco Research, USA.

I 103 CYTOLOGY OF HPV--HOW RELIABLE IS IT? Phillip J. Baird, Sydney, Australia

Human Papillomavirus (HPV) cannot be cultured in-vitro in the laboratory. Its detection is by indirect methods such as DNA hybridization, immunochemistry and cytopathic cellular changes.

Historically, cytology was the modality which highlighted the association of HPV and cancer of the female genital tract. Subsequently it has been used to screen populations for the presence of HPV infections and associated pre-cancerous changes. However, its specificity and sensitivity for HPV infection has been questioned in light of the ability of colposcopy, histology and DNA probes to detect the virus and the often poor correlation between various modalities.

This paper will review all the cytological features of HPV infection (up to 12 parameters) and correlate them with histologically proven HPV infections. It will be seen that some features are very specific but relatively insensitive, e.g. Koilocytosis. Recognition of the many minor cytological features of HPV infection will enable the Pap smear to remain a useful screening method.

I 104 TUMORIGENIC TRANSFORMATION OF CERVICAL EPITHELIAL CELLS IS ASSOCIATED WITH LOSS OF GROWTH INHIBITION BY TGF-β. L. Braun, S. Lauchlan, R. Mikumo, M. Gomez. Department of Pathology, Brown University. Transforming growth factor-β is a multifunctional protein synthesized by many different cell types both in vitro and in vivo. Whereas growth of cultured fibroblasts is often stimulated by TGF-β, this protein inhibits the growth of most untransformed epithelial cell types and a loss or decrease in the sensitivity of transformed cells to the antiproliferative effects of TGF-β is thought to be an important step in epithelial carcinogenesis. To address this issue we have compared the effects of TGF-β on the growth of primary cultures of normal cervical epithelial cells and the C4I cell line derived from an HPV-18 infected cervical carcinoma using autoradiography. We find that TFG-β inhibits serum-induced DNA synthesis of normal cervical cells in a dose dependent manner. In the absence of the growth inhibitor approximately 20% of the nuclei in individual colonies are labeled whereas exposure to increasing concentrations of TGF-β leads to an almost complete inhibition of DNA synthesis. On the other hand, no inhibition of DNA synthesis, cell proliferation or c-myc mRNA expression was observed in C4I cells even in the presence of 10 ng/ml TGF-β. In addition, C4I cells expressed higher levels of TGF-α mRNAs than normal cervical cells. Based on these findings we conclude that tumorigenic transformation of normal cervical cells is accompanied both by a loss of sensitivity to certain inhibitors of cell growth as well as an increase in expression of certain growth stimulatory proteins. Supported by USPHS grant # CA 46617

I 105 DETECTION OF E4 GENE PRODUCTS IN NATURALLY OCCURRING CONDYLOMATA ACUMINATA. Darron R. Brown, Robert C. Rose, Richard C. Reichman, Mark H. Stoler, and David G. Strike. Department of Medicine, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642

Proteins encoded by the E4 open reading frames (ORFs) have been identified in HPV1-infected plantar wart tissue, in HPV11-infected human foreskin implants grown in athymic mice, and by in vitro translation of mRNA from the CaSki cervical carcinoma cell line which harbors HPV18. The presence and level of expression of E4 gene products in naturally occurring condylomata acuminata lesions caused by HPV6 or HPV11 has not yet been established, although transcripts that could potentially encode E4 proteins have been identified. HPV6 and HPV11 are highly homologous, but the E4 region shows a comparatively low degree of sequence conservation relative to other ORFs. Thus antiE4 antibodies may show type specificity.

We have generated polyclonal antisera against the E4 gene products of HPV types 6 and 11 by cloning of E4 genomic DNA, expression of corresponding polypeptides in E. coli as beta-galactosidase/E4 fusion proteins, and immunization of rabbits. To determine if these antisera identify E4 proteins in a type specific manner, the E4 DNA fragments were also expressed in E. coli as TrpE/E4 fusion proteins and tested in Western blots. To ascertain if E4 gene products can be detected in genital lesions, tissue biopsies shown by Southern blots to contain HPV6 or HPV11 DNA were tested in Western blots using both rabbit antisera. Immunohistochemical stains were also performed on thin sections of biopsy specimens. Results of these studies will be presented.

I 106 TYPE-SPECIFIC HPV DETECTION IN PAP SMEARS BY IN SITU HYBRIDIZATION WITH ENZYME-DNA CONJUGATES, C.M. Bruns,[1] C.R. Ceccarelli,[1] J. Summerville,[2] T. Higgs,[1] and F.E. Taub,[1] of [1]Digene Diagnostics, Inc., Silver Spring, MD 20904 and [2]The Medical College of Virginia, Richmond, VA 23219.

Rapid and reproducible detection of HPV DNA sequences in routine cervical (Pap) smears and cervical cell suspensions spotted onto slides has been accomplished using non-radioactive DNA probes directly conjugated to horseradish peroxidase. No cross-hybridization is observed among probes for HPV types 16, 18, and 6/11 using a room temperature (20-25°C) hybridization. HPV sequences are detected in the nuclei of both immature and differentiated epithelial cells. The cell suspension samples exhibit lower background and better cell distribution than the routine smears, but were more tedious to prepare. Chemical pretreatment, fixation, enzymatic digestion, denaturation, hybridization, and amplified color development can be completed in under three hours. The use of a new method of signal amplification has greatly increased the sensitivity of the assay. Special multi-welled slides with Teflon borders allow numerous samples to be conveniently hybridzed with multiple probes in one run. Pretreatment of the slides has allowed firm cell adhesion throughout the processing steps, including steps effective in background removal. Microscopic evaluation of the permanent enhanced signal can be done at any time following color development.

I 107 **DESCRIPTIVE EPIDEMIOLOGY OF HPV INFECTION IN COLLEGE WOMEN,** Barbara J. Burkett, Christine M. Peterson, Brian E. Ward, Maggie Nuckols, Linda Birch, Constance Brennan and Christopher P. Crum, Departments of Pathology, Microbiology, Ob/Gyn and Student Health, The Univ. of VA Health Sciences Center, Charlottesville, VA 22908.

We evaluated the relationship of medical, behavioral and potential cofactors and genital papillomavirus (HPV) infection in the population. Subjects(N=532) were UVA students requesting routine Papanicolaou (Pap) smears. Five percent(n=28) were HPV+ by molecular hybridization analysis of exfoliated cervical cells and 2.4%(n=13) had an abnormal Pap smear. Of HPV+ cases 28.5%(n=8) had an abnormal smear. Associations of borderline significance with HPV+ included: decreased mean total time of chemical spermicide usage(p=.05), history of Trichomoniasis(p=.05), genital warts (p=.05), treatment with injectable progesterone, exposure of sexual partner to industrial dusts (p=.05), and increased mean number of sexual episodes per month (p=.05). Of marked significance were a history of previous abnormal pap smear (P<.001) and increased mean number of sexual partners in the 12 months prior to testing, despite the fact that the mean number of lifetime sexual partners in both groups was not significant. No association was found between HPV+, cigarette, alcohol, and contraceptive use. This study indicates that the cofactors governing HPV infection are complex, and that detection of HPV at any point in time may be influenced preferentially by more recent sexual events.

I 108 HUMAN PAPILLOMAVIRUSES (HPV) IN PREMALIGNANT AND MALIGNANT LESIONS OF IMMUNOSUPPRESSED PATIENTS. Chardonnet Y., Euvrard S., Guérin-Reverchon I., Viac J., Chignol M.C., Hermier C. and Thivolet J. INSERM U. 209, Clin. Dermatologique Pav R, Hop. E. Herriot 69437 Lyon Cedex 03 France

Immunosuppressed patients are known to develop numerous warts,keratoacanthomas (KA), Bowen's disease and squamous cell carcinomas (SCC). The signs of HPV infection were investigated comparatively on 66 cutaneous lesions taken from 16 renal transplant recipients (RTR), 3 cardiac transplant recipients (CTR), 4 hemodialyzed patients (HD) and 6 samples of normal skin from RTR, by histology of tissue sections, by indirect IF for the presence of group specific viral antigen, by in situ hybridization with biotinylated probes (HPV types 1,2,6,11,16,18) under stringent conditions and visualization of DNA-DNA hybrids with alkaline phosphatase for the presence of HPV DNA. 48/66 lesions showed histological signs of HPV. The 9 specimens of CTR and HD were HPV DNA and virus capsid antigen negative. In RTR 18/57 biopsies were HPV DNA negative; HPV types 1 or 2 were detected in scarse nuclei of 12 samples and type 16 was associated to 3 lesions; 24 biopsies reacted with more than one type. Viral capsid antigen was detected in 8 specimens (4 warts, 1 seborrehic wart, 1 SCC or KA, 1 KA and 1 dyskeratosis (DK). HPV type 2 was identified in these biopsies except in DK; moreover 3 warts and 1 KA reacted with HPV type 1 and less intensely with HPV type 18. The results obtained with in situ hybridization technique were compared with the Southern blot on available frozen tissues.

I 109 SCREENING OF CERVICAL SMEARS FOR HPV DNA IN SINGAPORE WOMEN BY FILTER IN SITU HYBRIDIZATION AND POLYMERASE CHAIN REACTION, Vincent Chow (1), K.M. Tham (1), M. Yeo (1), S.K. Lim-Tan (2), I. Sng (2), T. Thirumoorthy (3), and H.U. Bernard (1). (1) Institute of Molecular and Cell Biology, National University of Singapore, (2) Department of Pathology, Singapore General Hospital, (3) Middle Road Hospital, Singapore.
Our research goal is to determine the proportion of Singaporean women infected with genital HPVs using DNA hybridization assays of clearly defined sensitivity. Initially, we ascertained that nick-translated DNA probes (specific radioactivity $2x10^8$ cpm/ug) detect 0.1-1 pg of HPV DNA. These probes generate weakly detectable signals with 10^6 SiHa or C4-1 cells analysed by filter in situ hybridization, and intense signals with CaSki or HeLa cells. In the analysis of cervical smears, this sensitivity corresponds to about 10 productively infected cells (1000-10000 HPV copies each) or 1000-1000 tumor cells (10 HPV copies each). Applying this technique to scrapings from 740 women attending three family planning clinics in Singapore, 4% were found to harbour HPV, predominantly types 16 and 31. Furthermore, the analysis of smears from 130 prostitutes yielded an HPV positivity rate of 6.9%. Owing to the relatively low sensitivity of the test, and the narrow correlation of HPV presence with abnormal Papanicolaou cytology results (~25%), we have adopted the PCR technique, which permits us to detect single HPV DNA molecules among 10^5 cervical cells. This will thus represent an absolute rather than relative criterion for the diagnosis of HPV infection. The finding of a higher absolute infection rate may indicate that HPV infection is a necessary initiator of cervical neoplasia while other co-factors - ultimately determine the rate of cervical carcinogenesis.

I 110 HPV TYPING OF ADENOCARCINOMAS BY PCR; Richard Cone(1), Anna Beckmann (2), Denise Galloway (2), Jorma Paavonen (3), from (1) University of Washington, Seattle, (2) Fred Hutchinson Cancer Research Center, Seattle, (3) Helsinki University Central Hospital, Finland.

Paraffin embedded tissues from twenty cervical adenocarcinomas were retrospectively collected. Histopathologic examination documented that every adenocarcinoma was free from malignant squamous components. An unstained 6 micron section of each tumor was deparaffinized in xylene, scraped into an eppendorf tube and boiled for 10 minutes. The polymerase chain reaction (PCR) was performed with type specific primers representing part of the E6/E7 regions of HPV 6, HPV 16 and HPV 18. Results so far indicate that 4/20 (20%) of the adenocarcinomas contained HPV 6 and none contained HPV 16 (positive controls were included). Detection of HPV 18 using PCR is currently underway.

I 111 LOCALISATION OF HPV-16 DNA IN CERVICES OF WOMEN WITH CERVICAL EPITHELIAL NEOPLASIA BY PCR. Marion T.E. Cornelissen[1], Jan G. van den Tweel[2], Arie P.H.B. Struijck[3], and Jan ter Schegget[1]. 1. Dept. of Medical Microbiology, 2. Dept. of Pathology, 3. Dept. of Obstetrics and Gynaecology, Academic Medical Centre, University of Amsterdam, 1105 AZ Amsterdam, The Netherlands.

To study HPV-16 infection of the cervix uteri and the role of HPV-16 in the pathogenesis of (pre-)malignant cervical lesions we investigated the distribution of HPV-16 DNA in the cervix of women with CIN. To that purpose we utilized the PCR technique directly on sections of paraffin embedded cervical tissue (Shibata et al., J. Exp. Med. 167: 225-230) derived from conizations.

Oligonucleotide primers were selected complementary to a part of the E_7 and the L_1 ORF and after 32 amplification cycles PCR products were analyzed by agarose gel electrophoresis and Southern blotting. Reconstruction experiments to determine the detection level in paraffin sections are in progress. Simultaneously a paraffin section was examinded for the presence of the human beta-globin gene in order to control the PCR in this type of material. Depending on the size of the cone 6 to 10 paraffin embedded sections were analyzed from 7 HPV-16 DNA positive patients.

HPV-16 DNA was only detected in the sections that contained CIN lesions and/or koilocytes while no HPV-16 DNA was detected in section containing only normal epithelium, which is in accordance with our hypothesis that HPV-16 plays a causative role in the development of CIN lesions.

I 112 ACTIVATION OF THE HPV-16 P_{97} PROMOTER BY A KERATINOCYTE-DEPENDENT NUCLEAR FACTOR SHARED WITH CYTOKERATIN PROMOTERS

T.P.Cripe[1], A. Alderborn[2], T.H.Haugen[1], M.Lace[1], P.Bergman[2], U.Pettersson[2] and L.P.Turek, [1]VAMC and University of Iowa, Iowa City, IA 52242, and [2]University of Uppsala, Uppsala, Sweden

The transcriptional promoter of the human papillomavirus (HPV)-16 E6-E7 oncogene region, P_{97}, is differentially activated by cellular factors in uninfected human keratinocytes and cervical carcinoma cells which interact with a short 5' *cis* enhancer sequence (*Cripe et al., EMBO J. 6: 3745, 1987*). The minimal keratinocyte-dependent (*KD*) enhancer maps to three *cis* elements: (*i*) an activator protein-1 (**AP-1**)-binding site which further stimulates P_{97} transcripts in response to phorbol esters. However, the **AP-1** site as well as the adjacent glucocorticoid/progesterone receptor binding site (*Gloss et al., ibid 6: 3737, 1987*) can be deleted without inactivating the *KD* enhancer; (*ii*) a consensus CAAT-binding protein/nuclear factor-I (**CBP/NF-I**) site; and (*iii*) a consensus "cytokeratin 8-mer" (**CK**) site (AANCCAAA) of several human and animal cytokeratins and the human involucrin gene promoters. Both the **CBP/NF-I** and **CK** sites are required for the keratinocyte-dependent activation of the P_{97} promoter; all three elements are protected from DNase I digestion by nuclear proteins present in established, HPV-negative keratinocytes and cervical carcinoma cell lines (*Alderborn et al.*, this meeting). DNA binding experiments with multiple mutants of the **CK** element and its natural variants from cytokeratin promoters indicate that it binds a specific nuclear factor found in keratinocytes and cervical carcinoma cells, but not in fibroblasts or lymphoid cells, and that binding of the cognate factor *in vitro* correlates with transcriptional activation by the **CK** variants *in vivo*. We are currently purifying the **CK** factor and testing the role of the **CK** element in the transcriptional activity of cellular cytokeratin promoters.

I 113 THE RELATIONSHIP OF SMOKING TO THE INCIDENCE OF SQUAMOUS CELL VULVAR CANCER, Janet R. Daling, Karen J. Sherman, Joseph Chu, Fred Hutchinson Cancer Research Center and Department of Epidemiology, University of Washington, Seattle, WA 98195. To elucidate the role of smoking in the etiology of vulvar cancer, we interviewed 166 women with in-situ squamous cell vulvar cancer and 47 women with invasive squamous cell vulvar cancer. Their responses were compared to those of 24 women with non-squamous cell vulvar cancer and to the responses of 322 population-based controls selected through random digit dialing. Women with squamous cell vulvar cancer were more likely to be current smokers at diagnosis (in-situ 63%, invasive 40%) than women with non-squamous cell vulvar cancer (25%) or the random control population (27%). After adjusting for age and number of lifetime sexual partners, the risks for in-situ and invasive vulvar cancer among women who are current smokers relative to women who were not current smokers were 3.7 (95% CI = 2.3-5.9) and 2.0 (95% CI = 0.98-4.0), respectively. There was no increase in risk for non-squamous cell vulvar cancer associated with smoking, RR = 1.0 (95% CI = 0.4-2.3). Women with vulvar cancer who gave a history of genital warts were as likely to be current smokers at diagnosis as women with vulvar cancer who did not give a history of genital warts. Age at diagnosis did not modify the risk for vulvar cancer associated with smoking until after age 60, when the proportion of cases who were smokers dropped to 39% as compared to 23% of the controls. We hypothesize that the role of smoking in the etiology of vulvar cancer is in its effects in the immune system, and as the immune system deteriorates with age, the attributable risks associated with being a current smoker decline.

I 114 A CASE CONTROL STUDY OF CERVICAL DYSPLASIA IN A HIGH RISK POPULATION OF ALASKA NATIVE WOMEN. Davidson M, Parkinson A, Schnitzer P, Kiviat N, Bulkow L, Lanier A. Arctic Investigations Laboratory, Anchorage, Alaska; Harborview Hospital University of Washington, Seattle, Washington.

Alaska Native women have a higher and increasing incidence rate of cervical cancer compared to all U.S. Caucasians (O/E=2.3). The cases are 100 women with abnormal PAP smears showing dysplasia and a lesion confirmed by colposcopy. Control women are 100 age and race matched patients with a PAP smear showing no dysplasia. HPV DNA hybridization tests using a dot blot technique (Vira Pap and Vira Type, Life Technologies, Inc. Gathersburg, MD) were performed on all patients. Risk factors examined for dysplasia include the presence of HPV DNA, cigarette smoking, lifetime number of sexual partners, age at first intercourse, sexually transmitted diseases, oral and barrier contraceptive use, and pregnancy history. A multivarient analysis was performed to evaluate the strength of these risk factors and the results will be presented.

I 115 *THE COURSE OF CERVICAL DYSPLASIA RELATED TO HPV GENOTYPE.* *John R. Davis, Stefan Chipowsky, Thelma Whelchel, Michael Bernas and Earl A. Surwit, Departments of Pathology and Obstetrics-Gynecology, University of Arizona College of Medicine, Tucson, AZ 85724.*

A prospective study of moderate & severe squamous dysplasia of uterine cervix provided initial and interval follow-up biopsies (usually at 15 months). The cohort was enrolled in a retinoid clinical trial and was untreated by conventional lesion eradication modalities. Tissue sections from formalin-fixed biopsies were HPV typed by in situ hybridization using biotinylated viral probes 6, 11, 16, 18, 31 and 33. Forty of 64 cases were HPV positive (62.5%).

Preliminary classification was made into high risk (types 16 & 18), intermediate (31 & 33), and low risk (6 & 11). The high risk types were found in 33 cases (82.5%), intermediate types in 3 (7.5%), and low risk types in 4 (10%). These incidences correspond closely to those in the literature. The high risk types were more prevalent in severe dysplasia cases (89%) than moderate dysplasia (76%). Of particular interest were the 21 cases of moderate dysplasia at entry. Eight cases which remained moderate or progressed to severe were all associated with HPV-16-18, whereas only 8 of 13 (62%) cases showing regression had the high risk types. The study continues in follow-up and will eventually include 300 women. Identity of which cases were treated with retinoid vs. placebo will not be made until completion of the project.

I 116 THE EFFECTS OF THE NUMBER AND ARRANGEMENT OF E2 BINDING SITES ON E2-MEDIATED TRANSACTIVATION VARY BETWEEN HELA AND SW13 CELLS, Nathalie Dostatni, Françoise Thierry*, Rosemary Sousa, and Moshe Yaniv, Unité des Virus Oncogènes, Département de Biologie Moléculaire, Institut Pasteur, Paris, France. *Present Address, Lab of Tumor Virus Biology, NCI, NIH, Bethesda, MD 20892.

The product of the E2 ORF of papillomaviruses activates transcription through binding to a short palindromic DNA sequence ACCN6GGT. An interesting feature of this binding site is that it is always present in more than one copy in the regulatory regions of all papillomaviruses sequenced to date, although its relative positioning is not well conserved. In order to understand the relationship between the arrangement of binding sites and the level of transactivation by E2, these sites were cloned in varying numbers both 5' and 3' to the CAT gene driven by the TK promoter. Each construction was cotransfected with plasmid pC59, expressing the full length BPV1 E2 ORF, into HeLa and SW13 cells. Measurements of CAT activity revealed a copy number- and position-dependent effect of these binding sites on E2-mediated transactivation, with a synergistic effect seen for certain arrangements of two or more sites. However, the requirements regarding copy number and position were not identical in the two cell lines, one noticeable difference being the ability of E2 to transactivate in the presence of a single 5' site in SW13 but not in HeLa cells. These findings suggest that interaction of E2 with specific cellular factors may influence its transactivating capacity. The identification of these factors as well as the purification of the BPV1 E2 protein and the study of its properties *in vitro* are in progress.

I 117 DEMONSTRATION OF HUMAN PAPILLOMAVIRUS DNA IN PATIENTS WITH VULVITIS BY POLYMERASE CHAIN REACTION MEDIATED DNA AMPLIFICATION, Magnus Evander[1], Elisabeth Boden[2], Eva Rylander[2], and Goran Wadell[1], [1]Department of Virology, University of Umea, [2]Department of Obstetrics and Gynecology, University Hospital, S-901 85 Umea, Sweden

Many women are diagnosed as having vulvitis. The etiology of this condition has been uncertain. We usually do not detect HPV DNA in these patients with the Dot-Blot and Southern blot hybridization techniques. The polymerase chain reaction (PCR) offers the possibility to detect minimal numbers of HPV DNA in clinical specimens.

Biopsies from 10 women with diagnosed vulvitis were analysed for the presence of HPV DNA by the Southern blot technique and the polymerase chain reaction. Of these 10 women only one was weakly positive for HPV DNA by Southern blot, the type could not be determined. The other nine were negative for HPV DNA. The 10 women were then analysed with the PCR technique. Oligonucleotide primers, covering three regions of the HPV genome, the E1, E6 and E7 regions were constructed for detection of DNA sequences from HPV types 6, 16, 18 and 33. We were able to detect amplification products in 6 of the 9 women with vulvitis, which were negative for HPV DNA when analysed with the Southern blot technique. As a control, 8 Southern blot positive women, when analyzed with the PCR technique, were all positive for HPV DNA. Relevant negative controls were also included all through the PCR experiments.

These results imply that the papillomavirus genome is present at very low copy numbers in the lesions of vulvitis. To be able to demonstrate the presence of HPV DNA when the genome is rare, the PCR technique can be used.

I 118 CHARACTERIZATION OF HPV-16 AND 18 IN UTERINE CERVIX CARCINOMAS FROM TWO DIFFERENT MEXICAN POPULATIONS. 1Manuel L. González-Garay, 2Laura B. Avilés-Castro, 1Hugo A. Barrera-Saldaña and 2Patricio V. Gariglio. 1.ULIEG-UANL, Monterrey, N.L. 2.CINVESTAV-IPN Depto.de Genética, México, D.F. Human papillomaviruses (HPV) are probable etiological agents of cervical carcinomas. HPV types 16, 18, 31, 33 and 35 have been found in over 80% of all cervical tumors. In Mexico, about 35% of all malignant tumors in women are uterine cervix carcinomas, constituting their main cause of death. We had previously shown that in Mexico City, approximately 31% (5/16) of the analyzed tumoral samples contained HPV-16 DNA sequences (Cancer Cells 5, 1987). We have extended this observation in a comparative study including (Monterrey, Mexico City) and found that the prevalence of HPV-16 was similar in both populations. HPV-18 was detected in 20% of the invasive uterine-cervix carcinomas when analyzed at high stringency with probes containing either whole viral genome or relatively conserved regions of HPV-18 (pRB). However, only 7% of the tumors (1/14) were positive for probe pBB (containing highly specific LCR sequences), suggesting that the mexican populations contain a rather low proportion of HPV-18 sequences in uterine-cervix carcinomas. In both populations the majority of the samples analyzed (including the four stages of severity of the disease) contained integrated papilloma DNA sequences (5-10 copies). Other viral subtypes were found in 21 and 25% of the tumors Mexico City and Monterrey respectively.

I 119 GROUP SPECIFIC AMPLIFICATION OF HUMAN PAPILLOMAVIRUS SEQUENCES USING TWO UNIVERSAL PRIMERS. Lucie Grégoire(1,2), Maximillien Arella(3), Wayne Lancaster(4) and J. Campione-Piccardo(1,3,5). 1) Department of Microbiology and Immunology, University of Ottawa, Ottawa, Ontario, Canada, 2) Medical Laboratory, Ottawa Civic Hospital, Ottawa, Ontario, Canada, 3) Centre de Recherches en Virologie, Institut Armand-Frappier, Laval, Québec, Canada, 4) Department of Molecular Biology and Genetics, Wayne State University, Detroit, MI, U.S.A., 5) Molecular Virology Section, Laboratory Center for Disease Control, Ottawa, Ontario, Canada. The Polymerase Chain Reaction (PCR) procedure holds a large potential for the detection of human papillomaviruses (HPV) given the small amounts of viral genomic material usually present in pathological specimens. Also, new HPV types not causing any lesion may remain undetected. Universal primers may correspond to sequences highly conserved among HPV and therefore may be most appropriate for attempting the detection of these new types. Here we analyze the specificity of a pair of such universal primers for PV sequence amplification using PCR. We have already reported that the longest perfect homology among HPV sequences is a 12 nucleotide-long sequence (UNI sequence) within the first exon of the M-gene in the E1 region. A region of seven conserved aminoacids coded by the E1 ORF in all sequenced PV genomes allowed the detection of another highly conserved nucleotide region (WDO) about 850 nucleotides downstream of the UNI sequence. Two 21 nucleotide-long universal primers were synthetized containing the UNI and the WDO sequences. These two primers were used to successfully amplify sequences from all HPV types whose genomes were used as templates. The amplified material was shown in each case to be specific for HPV sequences within the E1 ORF. With clinical specimens the results correlate well with standard Southern hybridization. Also, we have detected a few cases positive by PCR which failed to prove positive by Southern hybridization. Using these reagents we hope to be able to demonstrate the presence of HPV sequences in specimens where the concentration of viral DNA is too low to be detected by conventional hybridization, as well as to be able to identify new HPV types.

I 120 A SIMPLIFIED SOUTHERN BLOT HYBRIDIZATION METHOD FOR TYPING HUMAN PAPILLOMAVIRUS, Dennis E. Groff and Jay George, Oncor Inc., Gaithersburg, MD 20877.
The association between HPV infection and cervical cancer is well established. We have developed a simplified Southern blot hybridization method to distinguish the major genital HPV types; thereby allowing the determination of low risk vs. high risk infections. By comparing nucleotide sequences, subgenomic regions were identified which exhibited minimum sequence homology between HPV types. Hybridization probes were made from these subgenomic regions. The specific probes were chosen such that hybridization to the homologous HPV produces one distinct band in Southern blots after digestion of cellular DNA with restriction endonucleases. The size of the hybridizing band is different for each HPV type. This allows the HPV type to be determined from a single band based on migration relative to known controls. Because of the simplified interpretation and the single hybridization step, multiple infections are easily determined. Results using this method on a series of biopsy samples from cervical and vulvar lesions and on cervical scrapes will be presented.

I 121 Sequence-specific and general transcriptional activation by the bovine papillomavirus-1 E2 *trans*-activator require an N-terminal amphipathic helix-containing E2 domain

T.H. Haugen[1], F.M. Mercurio[2], T.P. Cripe[1], B.J Olson[1], R.D. Anderson[1], D. Seidl[1], M. Karin[2], J. Schiller[3], and L.P. Turek[1], [1]VAMC and The University of Iowa College of Medicine, Iowa City, IA 52242, [2]University of California San Diego, La Jolla, CA 92093, and [3]Laboratory of Cellular Oncology, National Cancer Institute, Bethesda, MD 20892.

The sequence-specific *trans*-activator protein of bovine papillomavirus (BPV)-1, E2, strongly increases transcription at promoters containing papillomaviral *E2P* (ACC(N)$_6$GGT) *cis* motifs, but can also activate a wide range of cotransfected promoters without *E2P* cores to a lower extent. Analysis of multiple E2 mutants in transfected cells revealed that the C-terminal DNA binding E2 domain binds to the *E2P cis* sequences in the form of preexisting nuclear dimers. The DNA binding function of E2 was required for specific *trans*-activation of the *E2P* elements, as well as for the function of the previously described C-terminal 'short E2' *trans*-repressor. In addition to the C-terminus, specific *trans*-activation also required an intact N-terminal half of the E2 protein. When expressed alone, the N-terminal E2 domain was found to activate heterologous promoters without *E2P* elements to an extent comparable to *wild-type* E2, and therefore represents the functional transcription activation domain of the E2 factor. In contrast to other DNa+2.4Hbin ding. Its mechanism may thus involve protein-protein interactions between the N-terminal E2 domain which contains amphipathic helix motifs and common transcription factors at the promoter. To identify these cellular factors, we are currently testing multiple *cis* mutants in three minimal promoters for their response to E2 *trans*-activation.

I 122 ACTIVATION OF GM-CSF AND OTHER LYMPHOKINE GENES BY BPV E2 PROTEINS, Toshio Heike, Shoichiro Miyatake, Junji Nishida, Etsuko Abe, Takashi Yokota, Joseph Shlomai, Ken-ichi Arai, and Naoko Arai, Department of Molecular Biology, DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, CA 94304. T cells activated by antigen, mitogen or PMA/A23187 stimulation produce a battery of lymphokines. We reported that viral trans-activator, HTLV-I p40^{tax} as well as BPV E2, activates transfected lymphokine genes such as GM-CSF, IL-2, IL-3 and IL-4[(1)]. This activation which occurs both in T cells and fibroblasts, is upstream sequence dependent. The sequence required for sitmulation by BPV E2 localized between positions -95 and -73 (CLE2/GC element) where PMA/A23187 or p40^{tax} responsive element was mapped[(2)]. We concluded that E2 protein activates the GM-CSF gene by interacting with cellular component(s) in the signal transduction pathway without involving the E2 binding DNA motif (ACCN$_6$GGT), since no E2 binding consensus sequence was found in the CLE2/GC region and the activation of LCR by E2 proteins is unaffected by the addition of the CLE2/GC box sequence.

(1) Miyatake et al. (1988) Nucl. Acids Res., 16:6547.
(2) Miyatake et al. (1988) Mol. Cell. Biol., in press.

I 123 HIGHLY SENSITIVE, RAPID, AND SPECIFIC DETECTION OF HPV TYPES 6/11 AND 16/18 IN FORMALIN-FIXED PARAFFIN-EMBEDDED SECTIONS USING DIRECTLY CONJUGATED HRP-LABELED DNA PROBES, T. Higgs, N. Moore, G. Gamerman, C. Bruns, D. Badawi, and F. Taub, Digene Diagnostics, Inc., Silver Spring, MD 20904.

Probes that consist of horseradish peroxidase (HRP) directly conjugated to DNA have been previously shown to be effective in the detection of human papillomavirus (HPV) in histological sections. These probes allowed for the separation of HPV types 6/11 and 16/18 without detectable cross-hybridization. Since previous work showed HRP-DNA probes demonstrated an excellent signal-to-noise ratio, we attempted to increase the sensitivity of these probes by amplifying the nickel-modified diaminobenzidine (DAB) reaction product. The amplification of HRP-generated DAB precipitate by silver deposition is efficient enough to make initially sub-microscopic DAB deposits easily visible with routine light microscopy. In tissues shown to contain HPV, a 3- to 5-fold increase in the number of HPV-positive cells was observed after signal amplification. Even with the significant increase in sensitivity, no cross-hybridization can be detected between HPV6/11 and HPV16/18 probe cocktails. Thus, the apparent signal-to-noise ratio as well as the sensitivity has been greatly increased.

I 124 SELECTIVE SYNTHETIC OLIGONUCLEOTIDES FOR THE IDENTIFICATION OF HUMAN PAPILLOMA VIRUSES. Sophie Houard, Alfredo Cravador, Albert Herzog, Philippe Moureau, Marie J. De Vos, Ruba Carroll, Pierre d'Ippolito and Alex Bollen. Improbio, SA and Service of Applied Genetics, University of Brussels, rue de l'Industrie 24, B-1400 Nivelles, Belgium.
Among the various human papilloma viruses (HPV) found in mucosal or cutaneous epithelium, the occurence of certain types of viruses has been correlated to the development of carcinomas. It is therefore of crucial interest to characterize non ambiguously the type of HPV present in a lesion.
For this purpose, we have designed a computer program that allows us to compare simultaneously several long DNA sequences. This has enabled us to define specific oligonucleotide sequences for each of the viruses whose genomic sequences have been published.
Oligonucleotides corresponding to the sequences of HPV6b,11,16,18 and 33 were synthesized and their usefulness as (either radioactive or non radioactive) probes were investigated by *in situ* hybridisation with cervical tumor biopsies.
Primers for the polymerase chain reaction (PCR) were also determined and their specificity analysed with either cervical scrapes or tumor biopsies.
The hybridisation of HPV type-specific oligonucleotidic probes to the PCR amplification products appears to be at present one of the most selective and sensitive method for the detection of HPV in biological samples.

I 125 RAPID SEQUENCE ANALYSIS OF THE CAPSID REGIONS OF HUMAN PAPILLOMAVIRUSES USING SINGLE-STRANDED DNA GENERATED BY THE POLYMERASE CHAIN REACTION, Joseph P. Icenogle, Donna L. Miller, and Ruth Ann Tucker, Viral Exanthems and Herpesvirus Branch, Centers for Disease Control, Atlanta, Georgia, 30333. Asymmetric amplification using PCR has been implemented for the capsid region of human papillomaviruses using the procedures of Gyllensten and Erlich (PNAS, 85, 7652, (1988)). The single stranded DNA thus produced serves as an excellent template for sequencing reactions. Sufficient primer pairs have been identified to allow access to 76% of the region of the genome coding for capsid proteins. The primers are from relatively conserved regions of the genome, and amplify HPV 11, HPV 6, and HPV 16 containing samples efficiently and HPV 18 containing samples marginally. This technique allows at least 500 base pairs of information from 20 HPV containing samples to be determined per person per week. Sequence information on HPV's from both inside and outside the U.S. will be presented. The extent of sequence variation in the capsid region of these isolates will be discussed, as well as the potential utility of this technique for tracking strains in transmission studies.

I 126 DETECTION OF 5 HUMAN PAPILLOMAVIRUS DNAs IN 110 BIOPSY SPECIMENS FROM CERVICAL LESIONS BY IN SITU HYBRIDIZATION WITH SULFONATED PROBES. J. Jacquemier, F. Penault, O. Croissant, M. Dürst, P. Parc, B. Seradour, P. Meynard, P. Alfont and J. Hassoun. Institut J. Paoli – I. Calmettes –Laboratoire d'Anatomie Pathologique – 13273 Marseille cedex 9. France.
Since cervical lesions are particularly heterogenous, it is crucial to correlate HPV type with morphology. In situ hybridization is the only technique which can achieve such correlation. Biopsy specimens were taken under colposcopic control from 100 patients presenting lesions with significant cytologic changes of papillomavirus infection and/or dysplasia and 10 cases before curitherapy. Tissue samples were fixed in Carnoy. In 14 cases a second biopsy was obtained from the same area on the cervix and frozen at $-70^{\circ}C$ for Southern blot analysis. The DNA probes , HPV 6, 11, 16, 18 and 33 were sulfonated by chemical modification using the method of Sverdlov (PBS Organics, 67405 Illkirch, France). As control, PBR 322 plasmid was also sulfonated. Tissue sections were treated with a fresh solution of RNAse (Sigma) 10 μg/ml at $37^{\circ}C$. Next DNA probes and sections were denatured together in 50 % formamide at $70^{\circ}C$ for 30 minutes then hybridized at $42^{\circ}C$ for 18 hours at 0,2 μ g/ml of hybridization mixture. Then antibodies against the modified DNA labelled by alkaline phosphatase (AP) were applied . Analysis of the SB show that under 25 or 50 viral copies per cell equivalent, ISH was consistently negative. ISH failed to reveal HPV in any of the 29 metaplastic lesions studied. All the 6 condylomas presented HPV6 and HPV11. Of the 22 CIN1 , an oncogenic HPV was found in 87.2 % of cases with koilocytes associated with a papillary architecture (PA) as opposed to 57.1 % of cases with koilocytes alone. Of the 21 cases of CIN2, all 4 cases without koilocytes were negative, 56.25 % with koilocytes were associated with an oncogenic HPV. (HPV16 was the most frequent) . 9 of the 26 CIN3 contained HPV16 and 3 of the 10 infiltrating carcinoma. A non oncogenic virus was never found in CIN2 and CIN3. Oncogenic HPV is closely and significantly correlated with the number of mitoses. The mean number of mitoses exceeds 8 per field as early as the CIN1 stage. In our hands sulfonate probes proved to be easier to use than biotin probes. Our data also indicate that the incidence of oncogenic virus is higher at the onset of intraepithelial neoplasia : 68 % for CIN1 vs 34.6 % for CIN3. This suggests that at the initial stage the degree of replication is high while at stage CIN3 replication is lower possibly because the virus has already been integrated into the genome.

I 127 IN SITU HYBRIDIZATION WITH BIOTINYLATED PROBES IN DETECTION OF HPV INFECTIONS
Stina Syrjänen[1], Birgitta Andersson[2], Liisa Juntunen[2], [1]Department of Pathology, University of Kuopio, Finland, [2]Labsystems Research Laboratories, Helsinki, Finland.

The diagnostic value of in situ hybridization technique is well established in viral infections. The present study was undertaken to assess the application of a new rapid in situ hybridization tehcnique using biotinylated probes for identifying HPV infections in cervical biopsies and smears. In addition, three cell lines (HeLa, CaSki, SiHa), derived from cervical cancers and containing HPV DNA with known copy numbers, were also tested by using ^{35}S-labeled HPV DNA probes. The biotinylated hybride was detected with streptavidin-biotinylated-alkaline phosphatase complex using nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate as a substrate. Our results demonstrate that HPV typing can be reliably performed in one day with in situ hybridization using biotinylated probes. The in situ hybridization method with biotinylated probes is equally sensitive and specific as the one using ^{35}S-labeled probes, as judged from the intensity of hybridization signals obtained with the three cell lines. Our new method was compared with one commercial in situ hybridization kit based on biotinylated probes and the results were related to those obtained with dot blot hybridization.

I 128 AFFIPROBE - A MICROTITER PLATE TEST BASED ON SANDWICH HYBRIDIZATION IN SOLUTION. A. Kallio, T. Jalava and H. Söderlund. Orion Corporation Ltd., Orion Pharmaceutica, Genetic Engineering Lab. Valimotie 7, Helsinki, Finland.

The test is based on sandwich hybridization in solution followed by affinity based hybrid collection (Syvänen et al. NAR 14, 5037, 1986). The method uses two probes.One of them is the detector and the other the capture probe. The capture probe carries an affinity label enabling collection of the labeled hybrids onto a solid phase coated with the respective affinity pair. The affinity pair used is biotin/ streptavidin. Solution hybridization allows fast reaction kinetics and thus a one day test protocol.

We have now constructed a test suitable for use in a diagnostic laboratory. For that purpose two critical points were solved: i) The solid carrier is a microtiter plate enabling easy handling of the hybrids; ii) The label is ^{35}S permitting stable and reliable detection.

The test was optimized with DNA reagents cloned from the HPV16 DNA. Hybridization is for 3 hr followed by capture for 2 hr. Hybrids are eluted for scintillation counting which allows quantitative detection of the target DNA present in the specimen. The sensitivity of the test is 5 x 10^5 molecules of HPV 16 DNA. Parameters affecting test performance are presented.

I 129 ENZYMATIC AMLPLIFICATION AND CHARACTERIZATION OF HPV TYPE 16 MESSENGER RNA, Joo Yeun Kim, Chris Newell, John Lurain and Steven Wolinsky, Northwestern University Medical School, Chicago, IL

To understand the correlation between viral gene expression and tumorigenesis HPV type 16 mRNAs were examined using the polymerase chain reaction (PCR) technique. The PCR technique was used to enable the detection of rare viral transcripts and allow enzymatic amplification of specific regions of interest.

Primary human keratinocytes were transfected with plasmids containing HPV type 16 DNA. The stable transfectants were cultivated on collagen matrix rafts at the air-liquid interface to induce cellular stratification and differentiation. The established cell lines CaSki and SiHa, and cervical carcinomas, which harbor HPV 16 DNA were also studied. Total RNA was extracted from the transfected human keratinocytes and transformed cell lines. The viral mRNA species were enzymatically amplified following cDNA synthesis using an oligo-dT primer. To identify the various mRNA species, oligonucleotide primer pairs were designed based on the splice donor and acceptor sites and the specific open reading frames. The enzymatically amplified products were analyzed by Southern blot hybridization using an HPV type 16 genomic probe. Liquid hybridization was also performed using specific 32P-end-labeled oligonucleotide probes followed by polyacrylamide gel electrophoresis.

The PCR technique permitted the detection of various alternatively spliced early gene transcripts and allowed the identification of 3 different E6* messenger RNAs. DNA sequence data will provide further information about the various viral messenger RNA species associated with these transformed cell lines, cervical carcinoma cell lines and HPV 16-transfected primary human keratinocytes.

I 130 RISK FACTORS FOR HPV AND HSV-2 INFECTIONS IN GREENLAND AND DENMARK. A POPULATION-BASED STUDY. Susanne K. Kjær[1], Gerda Engholm[1], Ethel-Michele de Villiers[2] et al. [1]Danish Cancer Society, Danish Cancer Registry, Inst. of Cancer Epidemiology, Copenhagen, Denmark, [2]German Cancer Research Center, Heidelberg, Fed. Rep. Germany. Risk factors for genital HPV 16/18 and 6/11 as well as HSV-2 infections were investigated in a population-based study of 1600 randomly selected women (20-39 years) from Nuuk (Greenland) and Nykøbing F (Denmark). A total of 586 and 661 were included in Greenland and Denmark, respectively. All women had a personal interview and a gynecological examination with PAP-smear and cervical swab for HPV-analysis (FISH). Moreover, a blood sample was obtained for analysis of HSV-2 antibodies (ELISA). A significantly increased risk for HPV 16/18 was observed for abnormal smear (OR=2.3). Concerning HPV 6/11, factors as ever having had children (OR=2.0), increasing age at start of oral contraception (OR=2.3 for age $\geq$20 vs. never) and HSV-2 infection (OR=1.9) significantly increased the risk. Surprisingly, in view of the general concept of HPV as a sexually transmitted virus, women with "multiple" partners revealed a significantly lower risk for both HPV 16/18 (OR=0.6 for $\geq$20 partners vs. 0-4) and HPV 6/11 (OR=0.4 for $\geq$20 partners vs. 0-4) compared to those having "few" partners. In contrast, the risk for HSV-2 infection was significantly increased among women with early age at first intercourse (OR=2.3 for debut age $\leq$13 vs. 14-16 years), "multiple partners" (OR=2.6 for $\geq$20 partners vs. 0-4), and increasing number of sexually active years with unprotected cervix (OR=2.0 for $\geq$15 years vs. 0-9). Thus, the results of this study demonstrate a surprising risk pattern for HPV 16/18 and 6/11, but a pattern for HSV-2 in line with that to be expected for a sexually transmitted virus. This could indicate that HPV is transmitted not only by sexual contact, but misclassification problems of the filter in situ hybridization method cannot be excluded either.

I 131 **CHARACTERIZATION OF BPV-1 E2 REPRESSOR MUTANTS IN VIRALLY TRANSFORMED CELLS**
Paul F. Lambert and Peter M. Howley, Laboratory of Tumor Virus Biology, National Cancer Institute, Bethesda, MD 20892.

BPV-1 encodes two E2 repressor proteins which inhibit the activity of the E2 transcriptional transactivator. The major repressor species is a 31 kd protein generated from translation initiation at an internal E2 ORF methionine codon located just downstream of the P_{3080} promoter. The minor repressor species is a 28 kd species which is an E8/E2 fusion peptide generated from a 1234^3225 spliced mRNA. Previously, point mutations have been constructed and shown to disrupt the expression of either the major or minor E2 repressor genes (Lambert, Hubbert, Howley and Schiller, manuscript submitted). A detailed analysis of the biological consequences of these mutations on viral processes will be presented. Particular emphasis will be placed on the plasmid replication capacity of these mutant viruses. Disruption of the major E2 repressor gene results in a strong enhancement of viral replication, resulting in an increased plasmid copy number. This mutant also exhibited a slightly increased transformation capacity. No effect on viral copy number nor transformation capacity was observed for a mutant in which the minor E2 repressor gene was disrupted. A double mutant which lacks both E2 repressor gene appeared to lose both its capacity to transform and to replicate.

I 132 MUTATIONAL ANALYSIS OF THE M AND R GENES OF THE BPV-1 E1 ORF. Michael R. Lentz, Lauren Thorner, Nathan Bucay and Michael R. Botchan. Department of Molecular Biology, University of California, Berkeley, CA 94720.
We are investigating the structure of M and R, two BPV-1 genes involved in viral replication. Mutational analysis has located M, a negative modulator of replication, to the 5' one-third of the E1 ORF, while R, a positive replication factor, occupies the 3' two-thirds of E1. Our model predicts that a splice junction at nucleotide 1235 forms the boundary which separates the M gene from the R gene. To test this model, termination codons were engineered into the E1 ORF just 5' and 3' to the splice donor and acceptor nucleotides. Transformation efficiency is 10-20 fold down in these mutants and the viral DNA integrates into the chromosome. Experiments are currently under way to determine if these mutants can complement each other and known M- and R- mutants. In addition, the 5'-most ATG codon of E1 was mutated to ATA (Ile). This mutant also fails to replicate. Immunoprecipitation of wild-type BPV-1 transformed cells with anti-M antibodies shows a tight doublet under certain gel conditions at the predicted MW. The lower band in this doublet is missing in the initiator mutant. This suggests that there are two forms of the M protein, one that initiates at the 839 ATG of the E1 ORF and another that initiates upstream of E1. We are now attempting to complement the missing replication functions of the ATG- mutant with an M gene cDNA.

I 133 EPIDEMIOLOGY OF ORAL CANCER AND HUMAN PAPILLOMA VIRUS INFECTION
Christopher Maden, Audrey Christiansen, Anna Marie Beckman, Janet Daling. Fred Hutchinson Cancer Research Center,1124 Columbia St.,Seattle Wa,98104.
Sexually transmitted diseases have been implicated in the recently reported rise in incidence of oral cancer. To test this hypothesis, we are conducting a population based case-control study in three counties of Western Washington. The study is based on in-person interviews of male patients aged 18-65 with squamous cell cancer of the oral cavity and random digit dialed controls frequency matched to cases on age and county. A sample of exfoliated cells from the oral cavity of interviewed cases and controls is being analyzed for the presence of HPV 6 and 16 DNA by amplification using polymerase chain reaction, and Southern transfer hybridization with p^{32} labeled probes. Preliminary analysis of 62 cases and 29 controls adjusting for age and smoking has revealed, by interview, a prior history of the following: common warts, 61.3% cases,48.3% controls (OR=1.47,95% CI=.58-3.75);genital warts, 6.5% cases,3.4% controls (OR=1.42,95% CI=.15-13.8).
Recruitment of more cases and controls will allow further analysis of risk factors and adjustment for confounders.

I 134 HPV ANALYSIS BY SANDWICH HYBRIDISATION AND PCR, Alan D.B. Malcolm and Peter J. Nicholls, Department of Biochemistry, Charing Cross and Westminster Medical School, Fulham Palace Road, London, W6 8RF, U.K.
In order to facilitate large-scale screening of clinical samples, we have developed a sandwich technique involving covalent immobilisation of appropriate restriction fragments. Hybridisation of the sample to this, together with hybridisation of a labelled probe sequence allows a diagnosis to be made. The different strains may be distinguished by choosing appropriate restriction fragments. Specificity and high sensitivity can be achieved by using the polymerase chain reaction.

I 135 **CHARACTERIZATION OF THE DNA BINDING / DIMERIZATION DOMAIN SHARED BY THE E2 REGULATORY PROTEINS OF BPV-1,** **Alison A. McBride and Peter M. Howley,** Laboratory of Tumor Virus Biology, National Cancer Institute, NIH, Bethesda, Maryland 20892, USA.
At least three transcriptional regulatory factors are encoded by the E2 ORF of BPV-1. The full-length ORF encodes a transactivator and two repressor factors are expressed from the 3' half of the ORF. We have previously shown that the E2 transactivator contains an N-terminal domain important for the activation function and a C-terminal domain with a specific DNA binding activity. The C-terminal domain is also present in both repressors and E2 polypeptides containing this domain are able to form dimers. The DNA binding domain of the E2 polypeptides is not predicted to contain helix-turn-helix structures nor "zinc fingers" which are features characteristic of many other DNA binding proteins. The mechanism by which the repressors can inhibit E2-dependent transactivation is not yet known. Repression could result from direct competition for the E2 DNA binding sites by the positive and negative acting factors. Alternatively, subunit mixing between the transactivator and repressor species could give rise to heterodimers which are functionally altered or inactive. We are currently using site-directed mutagenesis to determine which amino acids in the C-terminal domain are important for the DNA binding and/or dimerization properties. A combination of immunoprecipitation and mutagenesis techniques are being carried out to determine the mechanism of repression in BPV-1 infected cells.

I 136 POLYMERASE CHAIN REACTION AMPLIFICATION OF HPV GENOMIC SEQUENCES IN THE HUMAN PROSTATE GLAND. P. McNicol,[1,2] D. Wasylyshen,[2] and J. Dodd.[3,4] Cadham Provincial Laboratory,[1] R3C 3Y1, Depts. of Medical Microbiology[2] and Physiology,[3] University of Manitoba, and Manitoba Institute of Cell Biology,[4] R3E OW3, Winnipeg, Canada. We used the polymerase chain reaction (PCR) procedure to amplify Human Papillomavirus (HPV) 16 and 18 sequences present in genomic DNA isolated from human prostate tissue, removed surgically for the treatment of urinary tract obstruction. Tissue underwent pathological examination was classified as benign prostatic hyperplasia (BPH) or carcinoma (CaP). Two oligonucleotide primers to conserved sequences within the long control region of both HPV types 16 and 18 were used in conjunction with Klenow polymerase to amplify a 50 basepair sequence unique to each virus type. Amplified sequences were resolved by agarose gel electrophoresis and detected visually and by Southern Blot hybridization to end-labeled HPV 16- or 18-specific oligonucleotide probes. To control for the specificity of amplification and detection procedures, we included in the reaction series a tube lacking DNA template, tubes containing 50 pg of HPV 16 or 18 genomic DNA, and a tube containing HeLa DNA template. Of the five CaP specimens examined to date, we detected HPV 16 DNA in three and HPV 18 DNA in one. Of the eight BPH specimens examined, HPV 16 DNA was detected in one. We examined two normal prostates removed at autopsy and did not detect HPV in either. Having confirmed the identity of the HPV types infecting prostatic tissue, we are synthesizing longer primer sequences to facilitate the use of Taq polymerase in our screening procedure.

I 137 HPV DETECTION IN CERVICAL SCRAPES BY THE POLYMERASE CHAIN REACTION AS COMPARED TO MODIFIED FISH AND SOUTHERN-BLOT ANALYSIS.
W. Melchers, E. Claas, P. Herbrink, J. Lindeman and W. Quint, Diagnostic Centre SSDZ, Department of Research and Development. P.O. Box 5010, 2600 GA Delft, The Netherlands.

The sensitivity and specificity of the PCR was determined to detect HPV in cervical scrapes from 80 women with a positive and 100 women, involved in a routine three yearly check up program, with a negative cytology result, and compared to results obtained from the modified FISH and Southern-blot analysis. To prevent amplification of cervical scrape contamination of HPV cloned sequences, type specific HPV 6, 11, 16, 18 and 33 "anti-contamination" primers, flanking the HPV clonings-sites were used. Because HPV 31 is not sequenced yet, no HPV 31 primers could be synthesized. The sensitivity of the modified FISH and Southern-blot was similar and the occurence of HPV in women with a positive cytology result and the control group was 46% and 2% respectively. Using PCR, 44 of the 68 (68%) patients with mild dysplasia, 6 of the 8 (75%) patients with severe dysplasia and all 4 patients (100%) with carcinoma in situ, were found postive for HPV DNA (overall occurence of 70%). In 18 HPV positive patients (21%), more than one HPV type could be detected by the PCR. In the control group an HPV occurence of 5% was found. These results further indicates that HPV is an important agent in the pathogenesis of cervical cancer.

I 138 INVASIVE CERVICAL CARCINOMA IN NATIVE AMERICANS OF THE SOUTHWEST: HPV ANALYSIS BY IN SITU HYBRIDIZATION,
I. David Mones, Charles R. Key, and Cosette Wheeler, Departments of Pathology and Cell Biology, University of New Mexico School of Medicine, and New Mexico Tumor Registry, Albuquerque, NM 87131.
Native American Indians of the Southwest have incidence rates for invasive cervical carcinoma which are approximately twice the national average. An ongoing prospective study of this same population has shown a 6% incidence rate for HPV with 88% of the HPV types belonging to types which have been associated with malignancies (HPV- 16/18/31/33/35). We analyzed formalin fixed paraffin embedded specimens from 75 Navajo and Pueblo Indians diagnosed with invasive cervical cancer (1965 thru 1987) for presence of specific HPV types. In situ hybridization was performed using a biotin-avidin, enzyme substrate reaction (ViraType in situ™, Life Technologies Inc.). Thirty-five percent of these cases were positive for HPV. Of the positive specimens 50% were HPV types 31/33/35 and 38% were HPV types 16/18. Similar in situ analyses of invasive cervical cancers from New Mexico Hispanics and non-Hispanic whites are currently underway.

I 139 HPV6b DNA TRANSFORMATION OF C127 MOUSE CELLS BY AN INDIRECT MECHANISM.
Don Morgan, Gene Pecoraro and Vittorio Defendi, Department of Pathology, New York University Medical Center, New York, NY 10016.

We have recently reported the in vitro transformation of C127 cells by HPV16 DNA (Cancer Res. 48, 2505-2511 (1988). We now present evidence supporting the transformation of C127 cells by HPV6b DNA. We transfected and G418-selected C127 cells to produce two cell lines, GC7-1 and GC11-9, each of which contain about 10 copies of integrated HPV6b DNA per cell at 1-2 passages after G418 selection. The cells did not exhibit striking morphological changes when compared to controls in monolayer culture, and did not readily grow in agar. This is in contrast to HPV16-transformed C127 cells, which form colonies in agar at elevated frequencies soon after G418 selection and initial passage in culture. At low passages (to passage 10), the HPV6b transfected cells were incapable of inducing tumors in nude mice; however, when higher passage cells (18 and up) were tested, the cells formed tumors in nude mice at high frequencies. Non transfected and pSV2neo-transfected C127 cells were negative for tumor formation during the assay. The levels of HPV6b DNA and RNA in the cells progressively diminished with increased passages, the viral genome becoming virtually non-detectable by passage 11 in the case of GC7-1 cells and in all GC7-1 induced tumors. Thus, HPV6b DNA, unlike HPV16, appears to act by an indirect mechanism, in which the viral genes are necessary for induction, but are not required for maintenance of transformation. Potential mechanisms being investigated include screening for induced cellular sequences which may play a role in HPV6b oncogenesis.

I 140 PREVALENCE OF HPV TYPE 16, 18 AND 11 IN CERVICAL SMEARS FROM PREGNANT WOMEN,
Ulla Hørding, Astrid K.N.Iversen, Johannes E. Bock, Bodil Norrild*,
DNA Tumor Virus Laboratory, Institute of Medical Microbiology, University of Copenhagen, Juliane Mariesvej 22, 2100 Copenhagen, Denmark.

To establish the prevalence of cervical HPV infection in a Danish population, we have analyzed cervical swabs from 1362 women, pregnant in the first and second trimester, by filter in situ hybridization. ^{32}P labeled probes of HPV DNA type 16, 18 and 11 were used. The over-all incidence of HPV was 8.8 % with a peak incidence of 13.5% in the group of women 19-22 years old. HPV 16 was found in 39% of smears from women who had cervical dysplasia at the examination (n=18), in 4.2% of women with earlier cervical dysplasia (n=71) and in 4.9% of smears from the remainder. HPV 18 sequences were found in 11.1%, 1.4% and 2.3% of smears from the three groups, and HPV 11 in 0%, 2.8% and 2.0% of smears from the same groups. The data seem to indicate that the HPV infection will resolve despite reinfection from the partner and probably not become latent. The results of analysis for HPV by PCR-technique of 200 additional smears from other pregnant women will be presented at the meeting.

*head of the laboratory.

I 141 THE BPV-1 E2 TRANSACTIVATOR IS EXPRESSED FROM MULTIPLE PROMOTERS.
Timothy Nottoli, Peter Vaillancourt, and Michael Botchan. Department of Molecular Biology, University of California, Berkeley, CA 94720. Unspliced RNAs which start downstream of P_{2443} can encode the full-length E2 gene product(Yang et. al., PNAS, 1985; Baker and Howley, EMBO, 1987). The E2 transactivator can be expressed from a cDNA construct, C59, which is unspliced and begins at nt 2360. Other RNAs have been described which initiate from upstream promoters P2 and P3 and which utilize the splice acceptor at nt 2558, and thus potentially encode the full-length E2 gene product. A cDNA derived from one of these(N15-2; Choe et. al., submitted) apparently initiates from P3 (located at nucleotide 890) and contains a splice from 1235 to 2558. When driven by a surrogate promoter, N15-2 transactivates transcription from a construct in which a fragment containing the P2 promoter plus its requisite E2-inducible enhancer elements is linked to the CAT gene.

We generated a point mutant of the splice acceptor at 2558, changing the AG|GT to CG|GT and presumably eliminating usage of this acceptor. Transfection of the complete BPV-1 genome containing this mutation, 2556C, reduced focus-forming ability when compared to wild type. Cotransfection of 1 µg of 2556C with increasing amounts of an E2 expression vector led to increased numbers of foci, demonstrating that E2 activity is reduced by this mutation and that at least one promoter upstream of 2443 is responsible for transcription of an RNA encoding the E2 product.

I 142 MAPPING OF HPV6 PROMOTERS BY TRANSCRIPTION IN VITRO, E. Offord, G. Hunter, M-C. Colomar and P. Beard, Swiss Institute for Experimental Cancer Research, 1066 Epalinges, Switzerland.
We are interested in the DNA sequences and proteins controlling human papilloma virus mRNA synthesis. We subcloned and purified the two fragments of HPV6b DNA generated by cutting with EcoRI (position 2188) and BamHI (position 4722). DNA fragments were transcribed *in vitro* using a nuclear extract of HeLa cells and RNA initiation sites estimated from specific run-off transcript lengths. Three regions functioning as promoters in this assay were identified. One corresponds to the TATA box identified at position 64 near the junction of the noncoding region and the "early" open reading frames. A second is 0.4 kb downstream from there. And the third, around position 4100, lies just upstream from the start of the L2 "late" open reading frame.

I 143 HUMAN PAPILLOMAVIRUS INFECTION OF THE CERVIX - THE RESULTS OF A PROSPECTIVE STUDY. Graham C.N. Parry[1], Micheline Byrne[2], Anne Morse[3], Dulcie V. Coleman[3], David Taylor-Robinson[4] and Alan D.B. Malcolm[1], [1]Biochemistry Dept, Charing Cross and Westminster Medical School, London, W6 8RF, U.K., [2]Praed Street Clinic, St. Mary's Hospital, Paddington, London, [3]Pathology Dept, St. Mary's Hospital Medical School, Paddington, London, and [4]Division of Sexually Transmitted Diseases, Clinical Research Centre, Harrow, Middlesex, U.K.
A possible role for the human papillomaviruses (HPV) in the etiology of cervical intraepithelial neoplasia (CIN) and cervical cancer has been extensively discussed over the past 10 years. Despite the accumulated evidence, the final proof of HPV as an etiological agent in cervical cancer is still not clearly established. A potentially useful method in the clarification of this issue is the use of prospective studies of patients diagnosed as having mild CIN lesions. Here we report on a prospective study of 100 women, diagnosed as having CIN I, who were followed by regular cytological and colposcopic examination over a 3-year period. At each visit a cervical scrape was taken for hybridisation studies and each woman screened for a wide range of microbiological infections. The results of all these tests are discussed and compared with the results of other studies.

I 144 ESTABLISHMENT OF GROWTH FACTOR INDEPENDENT LINES FROM HPV16-IMMORTALIZED HUMAN KERATINOCYTES. Lucia Pirisi (1), Ayse Batova (2), and Kim E. Creek (2), (1) Department of Pathology, University of South Carolina School of Medicine, and (2) Department of Chemistry, University of South Carolina, Columbia, SC 29208.
HPV16-immortalized human keratinocyte lines (HKc/HPV16d) were established in serum-free media by transfection of normal human keratinocytes (HKc) with a plasmid containing a dimer of the HPV16 genome. HKc/HPV16d lines maintain growth requirements typical of HKc. These include the requirement for epidermal growth factor (EGF) and bovine pituitary extract (BPE) for both clonal and mass culture growth. However, some subpopulations of HKc/HPV16d, but not HKc, exhibit the ability to grow in media lacking these growth factors (-GF medium). Growth factor independent (GFI) lines were visible as isolated colonies of slowly growing cells (1-5/100 mm dish) after 3 weeks of selection in -GF medium. These colonies grew slowly during the first 2 months in -GF medium, but their doubling time decreased progressively in the following 2 months to about 36 h, the same as the parental lines maintained in the presence of EGF and BPE. Attempts to develop GFI lines have been successful in all HKc/HPV16d lines examined (5 GFI lines were obtained from 5 different HKc/HPV16d lines), suggesting that the event(s) which produce the GFI phenotype must occur in a small proportion of the cell population in all the lines. Concentrated conditioned media collected from GFI lines stimulated thymidine incorporation in growth factor dependent keratinocytes, suggesting the secretion of an autocrine growth factor. The identification of this growth factor, as well as the expression of specific HPV16 sequences in the GFI lines are currently under investigation.

I 145 DETECTION OF HPV IN URINE SAMPLES OF MALE PATIENTS BY THE POLYMERASE CHAIN REACTION. W. Quint, W. Melchers, R. Schift, E. Stolz, and J. Lindeman, Diagnostic Centre SSDZ, Department of Research and Development, P.O. Box 5010, 2600 GA Delft, The Netherlands.

To investigate HPV transmission by males, HPV detection was performed by the Polymerase Chain Reaction (PCR) on urine samples of 17 male patients with Condylomata acuminata in the meatus urethrae. As a control group urine samples from 20 male laboratory co-workers were analyzed. The DNA was extracted and purified from 50 ml urinesediments and subjected to 40 cycles of amplification with HPV 6 and HPV 11 specific anti-contamination primers and the heat stable Taq-polymerase. HPV was detected in the urines of 13 (76%) males from the patient group. Seven patients were found positive for HPV 6 DNA, five patients for HPV 11 DNA, one patient was found positive for both HPV 6 and HPV 11 DNA and in four patients no HPV 6 or HPV 11 DNA was found. In none of the males of the control group HPV 6 or HPV 11 could be detected. Since HPV can be transported by the urine flow, probably by an easy exfoliation of the HPV infected cells, transportation by the semen flow during ejaculation may also be possible, implicating a direct implantation of HPV in the cervix and a female HPV infection.

I 146 AFFIPROBE FOR DETECTION OF HPV DNA FROM CERVICAL SCRAPES. M. Ranki and J. Raussi. Orion Corporation Ltd, Orion Pharmaceutica, Genetic Engineering Laboratory, Valimotie 7, Helsinki, Finland.

AffiProbe-test was constructed in our laboratory for rapid detection and typing of HPV 6/11, HPV 16 and HPV 18 from crude clinical specimens. We have previously used the solution hybridization for detection of HPV 16 DNA from cervical scrapes of patients with premalignant lesions belonging to a follow up study (Parkkinen et al. Mol. Cell. Probes, in press). The test was shown to have a sensitivity comparable to that of in situ hybridization. It also detects infections with a low copy number of viral DNA per cell provided representative samples are collected.

For this study asymptomatic patients were selected on the basis of a positive HPV DNA finding , but very often with normal cytology. Separate scrapes from the cervix and the vaginal epithelium were collected to study the optimal specimen. The specimens were divided for testing the above HPV types. The control test was a commercial dot blot test. The AffiProbe test is fast, the results are obtained within the same day. The results are numerical values allowing quantification of the target DNA. The test is insensitive to biological impurities normally occuring in the scrapes. Thus, no pretretment for DNA purification is required. Results of the clinical study will be presented.

I 147 EPIDEMIOLOGICAL EVIDENCE FOR AN INTERACTION BETWEEN ORAL CONTRACEPTIVE USE, HPV INFECTION, AND INVASIVE CERVICAL CANCER, Allan Hildesheim, Louise A. Brinton, William C. Reeves, Carol Lavery, Maria M. Brenes, Maria E. de la Guardia, and William E. Rawls, Environmental Epidemiology Branch, National Cancer Institute, Bethesda, MD 20892. The distribution of cervical cancer risk factors in women testing positive and negative for HPV DNA were compared among 197 cases of histologically confirmed invasive cancer and 374 normal controls. Sixty-one percent of cases were found to be positive for HPV as detected by Southern and slot-blot hybridization of DNA extracted from biopsy tissue. Comparing positive and negative cases with all controls using polychotomous logistic regression, HPV infection was found to be an independent risk factor for cervical cancer from smoking, multiple pregnancies, and early age at first intercourse. Differences in risk between the two groups were detected only for oral contraceptive (OC) use. OC use was a risk factor for cervical cancer in the positive group (OR=2.1; 95% CI=1.0,4.6; 4+ yrs Vs. never users), and slightly protective in the negative group (OR=0.6; 95% CI=0.2,1.7). This suggests either a true interaction between HPV and OC use or increased detectability of HPV DNA in OC users.

I 148 **THE MODULATION OF HPV-16 TRANSCRIPTION BY TRANS-ACTING VIRAL GENE PRODUCTS,**
Helen Romanczuk, Karl Münger, William C. Phelps, and Peter M. Howley, Laboratory of Tumor Virus Biology, National Cancer Institute, Bethesda, MD 20892.
The presence of HPV-16 DNA in a high percentage of cervical cancers, combined with the capacity of the DNA to immortalize primary human squamous epithelial cells and to cooperate in the transformation of primary rat cells, strongly suggest that HPV-16 plays a role in carcinogenic progression. The HPV-16 E6 and E7 genes, which apparently mediate these immortalization and transformation functions, are expressed in cervical cancers. In order to study the transcriptional regulation of these viral genes, we have analyzed elements in the HPV-16 long control region (LCR) that are likely to contribute to the expression of E6 and E7 in human cervical cancer cells and in primary human epithelial cells. We have compared the activity of the LCR in these cells alone and in the presence of HPV-16 E6, E7 or E2 gene products. In addition, we have generated mutations in the LCR sequences which are normally responsive to papillomavirus E2 gene products in order to determine the importance of these sequences in transcriptional regulation.

I 149 THE INTERACTION OF CELLULAR FACTORS WITH THE NCR OF HPV16
Gary J. Sibbet and M. Saveria Campo. Beatson Institute, GLASGOW.
The progression of HPV16 associated CIN to cancer is frequently accompanied by chromosomal integration of the viral genome. The cell line SiHa, derived from a cervical SCC, contains a single integrated copy of HPV16. We have mapped a complex pattern of nuclease hypersensitive sites at high resolution in SiHa chromatin to the tissue specific enhancer within the HPV16 NCR. DNaseI footprinting (FP) of cloned DNA *in vitro* confirms the presence of cellular trans-acting factors (CTAFs) in SiHa nuclear extract with binding specificity for at least 13 sites in the HPV16 enhancer. The majority of FPs contain the consensus T/AGGCT/A, which is analogous to putative cytokeratin and CAAT box motifs. However the bound CTAFs found in nuclear extracts of SiHa as well as CaSki and HeLa are also found in K562 and MRC5 cells and appear to be related to C/EBP but not to NFI/CTF, NFY or NFY*/CRF. We have also observed the binding of additional CTAFs to the HPV16 enhancer. Their role in viral expression and tissue specificity will be discussed.

I 150 The Association Between Pregnancy and HPV Prevalence. Smith EM[1], Johnson SR[1], Jiang D[1], Anderson RA[2], Turek LP[1,2]. [1]Univ. of Iowa, [2]VAMC, Iowa City, IA 52242.
This case-referent study examined the effects of pregnancy on the prevalence of HPV infection, comparing 69 pregnant and 54 nonpregnant age-frequency matched controls. Patients were identified in an Ob/Gyn clinic at the university, at a private practice, and a Family Planning clinic. All subjects completed a questionnaire regarding data about demographic and cervical cancer risks. Additional data for each patient included a medical record of relevant conditions and dates, a detailed cytology review, and HPV DNA specimen results of status (positive/negative) and typing. DNA hybridization was performed using Southern blot and the ViraPap/ViraType dot blot kit.

Bivariate and multivariate analyses were performed with HPV status as the dependent variable and patient status (pregnant/nonpregnant) as a variable included in each regression model. Results indicate that there were no significant differences between HPV+ and HPV- groups on pregnancy status, age, number of sex partners, age of first intercourse, average amount of intercourse per month, number of pregnancies, or smoking status, including dose, duration, or age initiated. Only a current Pap result of cervical dysplasia (OR=8.99, CI:1.5, 54.9; α=.05, 95% CI) was a significant predictor of HPV status, whereas patient group was not (OR=3.05, CI:0.4, 25.3). HPV 16/18 and 31/33/35 were identified with almost equal frequency. The prevalence of HPV among pregnant women was 14.8 vs. 14.5% among nonpregnant women. Additional findings from the 4 data sources will be described.

I 151 PRESENCE AND EXPRESSION OF HPV6 IN A SQUAMOUS CELL CARCINOMA OF THE LUNG, Bettie M. Steinberg, Teresa DiLorenzo, Allan Abramson and Tim Crook, Dept. of Otolaryngology, Long Island Jewish Medical Center, New Hyde Park, NY 11042 and Imperial Cancer Research Fund, Dept. of Pathology, Univ. of Cambridge, Tennis Court Rd., Cambridge CB21QP, Eng. A 26 year old male with a 24 year history of laryngeal papillomas, containing HPV6a, which extended into the trachea and lungs, developed a squamous cell carcinoma of the lung. The carcinoma contains 50-100 copies of HPV6 DNA per cell. Restriction digests with Bam HI and Eco RI each yield 2 fragments. PstI digestion shows a shift in molecular weight of the 1496 and 1774 bp bands when compared to HPV6a from a laryngeal papilloma from the same patient. This data suggests a possible duplication or rearrangement in the upstream regulatory region. Digests with XhoI, KpnI and SalI, which do not cut HPV6a, are consistent with integration. cDNA clones reveal several large transcripts. The pattern of HPV transcription is currently being analyzed.

Supported by grant 2PO1NS19214 from the National Institute of Neurologic and Communicative Disorders and Stroke, and by a grant from the Irving Schneider Family Foundation (BMS and TD).

I 152 THE BPV E2 PROTEIN BLOCKS THE ACTIVITY OF A CELLULAR FACTOR REQUIRED FOR TRANSCRIPTION, BY BINDING TO AN OVERLAPPING SITE. A. Stenlund and M. Botchan. Department of Molecular Biology, U C Berkeley, Berkeley CA 947 40.

The BPV P1 promoter has an unusual structure with a major portion of its regulatory sequences located downstream of the cap site. A region that is absolutely required for transcription both *in vitro* and *in vivo* is located in a 28 nt sequence immediately downstream of the cap site. This sequence also contains the recognition sequence for a putative binding site for the viral transfactor E2. Co transfection of the P1 promoter with with an E2 expression construct leads to suppression of transcription from the P1 promoter. This is in contrast to several other BPV promoters which are activated by the E2 gene product. We will show that this repression is caused directly by E2, since repression can be reproduced*in vitro* with E2 polypeptide produced in E.coli and also that binding is required since mutations that destroy the E2 recognition sequence lead to loss of repression.

I 153 USE OF THE POLYMERASE CHAIN REACTION TO DIAGNOSE RELATED PAPILLOMAVIRUS INFECTIONS IN ARCHIVAL CERVICAL SPECIMENS, Jay Stoerker, Anil Sood, Anne Hackenewerth, James B. Allen, Sheila S. Reilly, and Jared N. Schwartz, Department of Biology, University of North Carolina at Charlotte, Charlotte, North Carolina 28223. A library of formalin fixed and paraffin embedded cervical samples from patients undergoing endoscopy was assayed for the presence of human papillomavirus (HPV) DNA by low stringency dot blot hybridizations with genomic clones of HPV types 6 and 11. Those samples which were positive were additionally assayed by high stringency hybridizations using asymetric riboprobes prepared from nonconserved regions of HPV types 6,11,16,18 and 31, and unique oligomeric probes from types 6, 16 and 18. These results were compared to Polymerase Chain Reaction (PCR) analysis on the same samples. The PCR results were less equivocal than the hybridizations, and agreed better with the oligo probe analyses than did the riboprobes. Distinctions between types which could be made only with difficulty by varying conditions of hybridization and washing were made in a straightforward manner with PCR. These findings indicate that accurate risk evaluation with regard to HPV type can be made from cast-off clinical materials, and may ultimately be done on Pap smears after histologic examination.

I 154 DETECTION AND EXPRESSION OF HUMAN PAPILLOMAVIRUSES IN ADENOCARCINOMA IN SITU OF THE UTERINE CERVIX, Mark H. Stoler, Cheryl Rhodes, April Whitbeck, Colin R. Laverty*, Thomas R. Broker and Louise T. Chow, *Eastwood, Australia and Departments of Pathology and Biochemistry, University of Rochester Medical Center, Rochester, NY 14642
Adenocarcinoma of the cervix accounts for between 10 & 30% of cervix cancer and the frequency seems to be increasing. Unlike squamous carcinoma of the cervix, there has been relatively little information about the pathogenesis of these tumors. Recently several reports have described the frequent presence of human papillomavirus (HPV) type 16 and type 18 DNA in cervical adenocarcinomas. The spectrum of histologic precursors of adenocarcinoma are not as well defined as for squamous cancers with the exception of adenocarcinoma in situ (ACIS), which is frequently found associated with invasive adenocarcinoma and is considered to be the immediate histologic precursor of these cancers. Biopsies of ACIS frequently have a coexisting squamous cervical dysplasia (CIN). While the majority of CINs contain HPV-DNA, there is comparatively little information about ACIS. We performed in situ hybridization for HPV messenger RNA expression using antisense RNA probes for HPV types 6, 11, 16, 18 & 31, on biopsies from 21 patients with a histologic diagnosis of adenocarcinoma in situ. One patient had only residual CIN-3 in the block and this expressed HPV-18. Of the 20 remaining cases, 5 also had histologic evidence of invasive adenocarcinoma and 6 had coexisting CIN. 18/20 of the cases of ACIS expressed HPV mRNA. There were 12 cases of type 18 and 6 cases of type 16. In the associated squamous dysplasias, 4 expressed HPV 16 and 2 expressed HPV 18 mRNA. In the cases with invasive adenocarcinoma 3 were type 18 and 2 were type 16. One patient had a mixed infection with HPV type 16 in the squamous lesion and HPV-18 in the ACIS. All 5 of the invasive cancers expressed the same viral type as in the patient's ACIS. A subset of these lesions have been subjected to a more detailed analysis using exon specific probes for each of the major HPV open reading frames. In situ hybridization for HPV mRNA expression has detected HPV gene expression in 19/21 (90%) of biopsies from patients with ACIS of the cervix. The ratio of HPV type 18:16 is approximately 2:1. The continuity of viral type between precursors and invasive lesions supports the concept that human papillomaviruses are also involved in the pathogenesis of cervical adenocarcinoma.

I 155 **PROSPECTIVE FOLLOW-UP OF GENITAL HPV INFECTIONS: LIFE-TABLE ANALYSIS OF HPV TYPING DATA, Kari Syrjänen, Vesa Kataja, Stina Syrjänen, Merja Yliskoski, Sinikka Parkkinen, Seppo Saarikoski, Rauno Mäntyjärvi, Martti Väyrynen, Jukka T. Salonen, and Olli Castrén,** Department of Community Health and General Practice; Department of Clinical Microbiology; Department of Gynecology & Obstetrics; Department of Pathology, University of Kuopio, POB 6, SF-70211 Kuopio, Finland.
A series of 532 women with an established genital HPV infection has been prospectively followed-up (without treatment) since 1981 for a mean of 50 (SD 21) months. The patients were examined by colposcopy, PAP smears and/or punch biopsy every 6th month. HPV typing of all biopsies was completed using in situ, Southern blot and/or sandwich hybridization with DNA probes of HPV types 6,11,16,18,31 and 33. The life-table method was applied to analyse the clinical course (i.e. spontaneous regression and clinical progression) of the HPV lesions, stratified by their HPV typing data, currently available from 341 women. Clinical progression was significantly associated with the HPV types present in the lesions. Progression rate was only 7.5% (3/40) for HPV 6 lesions, as contrasted to 14.3% (6/42) for HPV 11, 35% (28/80) for HPV 16, 11.1% (3/27) for HPV 18, 20% (5/25) for HPV 31, and 20% (5/25) for HPV 33 lesions. The lowest progression rate, 4/91 (4.3%) was established for lesions which remained constantly HPV DNA-negative. The probability of progression varied significantly between the six HPV types (p=0.0012). On the other hand, HPV type was not of predictive value for spontaneous regression (p=0.8044 overall). The value and limitations of histological grade, PAP smear findings, and HPV type as prognostic factors of HPV infections are discussed.

I 156 LOCALIZATION OF THE MAJOR LATE PROMOTER (P_L) IN HUMAN PAPILLOMAVIRUS TYPE 1a, Marshall E. Tolbert and David J. Giron,Department of Microbiology and Immunology, Wright State University, Dayton, OH 45435. Late gene (L_1, L_2) of Papillomaviruses (PVs) appears to be restricted to the upper, differentiated keratinocyte layers of productively infected tissue. The regulation of this region, whether viral or cellular, remains unclear. Likewise, the location of the major late promoter (P_L) is unknown for most PVs. We have attempted to localize the P_L of Human Papillomavirus Type 1a (HPV-1a), a benign virus implicated in palmar and plantar warts. A late region fragment spanning base pairs 6484 to 7496 was obatained by digesting the viral genome with the restriction enzyme Sca I. This fragment was then cloned into the promoter probe vector pSVOcat. The recombinant vector was then transiently transfected into CV-1 (green african monkey kidney epithelial) cells via electroporation. Four days post-transfection, promoter activity was assayed by induction of chloramphenicol acetyl transferase (CAT). We were able to demonstate promoter activity within the Sca I fragment. The data suggests that the location of the HPV-1a P_L corresponds with the location of the P_L in Bovine Papillomavirus (BPV-1).

I 157 INFLUENCE OF GLUCOCORTICOID HORMONES ON HPV 18 EARLY GENE EXPRESSION AND THE DIFFERENTIATION PATTERN IN CERVICAL CANCER CELLS, Magnus von Knebel Doeberitz, Heiko Drzonek and Harald zur Hausen, Institute for Virus Research, German Cancer Research Center, Im Neuenheimer Feld 506, 69 Heidelberg, FRG

Steroid hormones have been implicated to play an important regulatory role on HPV early gene expression. The influence of these hormones on HPV 18 gene expression in cervical cancer cell lines was investigated. In C4-I and C4-II cells glucocorticoid hormones lead to enhanced HPV 18 early gene expression being accompanied by an increased proliferation rate. In HeLa cells, despite the presence of functional glucocorticoid receptors no alteration of HPV 18 gene expression and the proliferation pattern is observed. In SW 756 cells however, glucocorticoids lead to a strong decrease of HPV 18 gene expression which is associated with severe alterations of the proliferation and differentiation pattern of these cells. Thus, specific cellular factors are involved in the steroid hormone mediated effect on HPV 18 positive cervical cancer cells differing among cells of heterogenous origin. However, alteration of HPV 18 early gene expression is associated with important consequences for the biological properties of these cervical cancer cells.

I 158 APPLICATION OF ANTICONTAMINATION PRIMERS FOR THE DETECTION OF HUMAN PAPILLOMAVIRUS IN CERVICAL SCRAPES. A. v.d. Brule, C.J.L.M. Meijer, E.K.J. Risse, Th. Helmerhorst and J.M.M. Walboomers. Department of Pathology, Free University Hospital, De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands.

Human Papilloma Virus types (HPV) have been implicated as etiological agents in cervical cancer. To test the presence of HPV types in cervical scrapes the polymerase chain reaction (PCR) was used and compared with the results of a combined modified filter in situ hybridization and dot blot procedure. In order to avoid detection of HPV plasmids "anti-contamination" primers flanking the HPV cloning sites were used in the PCR to detect HPV--6, -11, -16, -18, -31 and -33. The results showed that the PCR method was superior to the other detection method.

Next a low (n=1240) and a high risk group (n=403) for the development of cervical cancer was screened for HPV genotypes with the optimalized PCR technique. It appears that the overall prevalence of HPV in cervical scrapes of the high risk group without cytological changes was 12% while in the low risk group 5% was found. In smears with dysplastic or neoplastic changes the prevalence of HPV was 40 and 70 percent respectively in the low and the high risk group.

These data obtained with the optimalized PCR technique indicate an important role of HPV detection in the screening of cervical scrapes to identify women for an increased risk for the development of cervical cancer.

I 159 **BPV-1 CONTAINS A SPECIES SPECIFIC CONSTITUTIVE ENHANCER THAT IS NEGATIVELY REGULATED BY THE E2 REPRESSOR**, Scott B. Vande Pol and Peter M. Howley, Laboratory of Tumor Virus Biology, National Cancer Institute, Bethesda, Maryland 20892.

A fragment of BPV-1 from the LCR (nt 6958-7386) strongly activates the SV40 early promoter. The enhancer fragment is active in primary bovine fibroblasts and primary bovine epithelial cells. It is very weakly active in mouse C127 or mouse L cells, and is inactive in monkey CV-1 cells. Deletion mapping of the fragment shows most of the enhancer activity to map between nt 7162-7275; this smaller fragment contains a single binding motif for the E2 gene product at nt 7203. Expression of the E2 repressor negatively modulates the enhancer activity of the 7162-7275 fragment in primary bovine cells. Mutations were constructed within the E2 binding site (ACCN$_6$GGT) at nt 7203 that both disrupted and did not disrupt E2 binding; both categories of mutations decreased enhancer activity. Mutations in an adjacent CCAAT motif (nt 7196) and immunoglobulin promoter element (nt 7216) did not depress enhancer activity. Thus, there is genetic evidence for a species specific constitutive enhancer factor which overlaps with the 7203 E2 binding site. The possible functional roles of this enhancer will be discussed.

I 160 MOLECULAR ANALYSIS OF GENITAL PAPILLOMAVIRUSES IN LATENT OR SUBCLINICAL INFECTIONS, Aldo Venuti^*, Alfonso Sedati~, M. Saveria Campo* and Maria L. Marcante^, ^Laboratory of Virology and~ Department of Gynecology, Regina Elena Institute for Cancer Research, 00185 Rome, Italy, *Beatson Institute for Cancer Research, Glasgow G61 1BD, U.K. There is evidence that a substantial percentage of genital papillomavirus infections are subclinical. During a mass screening of a female population (800 women) for the presence of HPV infection we obtained hybridization of an HPV 16 and 18 DNA mixture to cervical scrapes of subjects without any cytological and/or clinical evidence of disease. These women were enrolled in a follow-up program to ascertain the risk of genital cancer development. Every four months women were colposcopically examined and swabs were collected for cytological and bio-molecular analysis. Viral DNA and mRNAs were tested by Dot-blot, Southern-blot and PCR techniques. At the present time a regression of subclinical infection is observed in 40% of patients. Of the remaining 60%, 20% developed clinical and/or cytological features of virus infection and 80% still remain at the stage of latent infection. Results of this follow-up and the analysis of the physical state of the viral genome and its RNA expression in these subclinical infections will be reported. Work partially supported by Associazione Italiana Ricerca sul Cancro/88 and by a grant of Ministero della Sanita' Italiana/88.

I 161 HUMAN PAPILLOMAVIRUS DNA IN LARYNGEAL PAPILLOMATOSIS DEMONSTRATED BY IN SITU HYBRIDIZATION, Luisa L. Villa and Jose E. Levi, Ludwig Institute for Cancer Research, São Paulo, 01509, Brasil.
The presence of human papillomavirus (HPV) DNA in laryngeal papillomatosis has been already demonstrated by the DNA hybridization technique. However, even though an association between the juvenile-onset of laryngeal papillomatosis and maternal genital tract HPV infections has been suggested, the epidemiology of such lesions is far from being completely understood. It is also important to consider the adult-onset of laryngeal papillomas, that very rarely exhibit signs of intraepithelial dysplasia and malignant conversion. Such dysplastic papillomas and carcinomas frequently harbor HPV DNAs of other types than the common HPVs 6 and 11 found in the juvenile lesions. We are interested in demonstrating the presence of various types of HPV in different laryngeal abnormalities, ranging from the benign juvenile papillomatosis to invasive carcinoma of the larynx. Fixed, paraffin-embedded tissue is submitted to in situ DNA hybridization, using as probes the radiolabelled DNAs of HPVs 6, 11, 16 and 18. In a series of 30 papillomatosis, we have found a very high prevalence of HPVs 6 and 11. Type-specific HPV DNAs in each lesion is determined by amplification of a 450 bp fragment in the L1-URR region of the HPV genomes directed by the polymerase chain reaction. Our data are in agreement with the concept that HPV is implicated in the etiology of laryngeal papillomatosis.

I 162 THE DIAGNOSTIC VALUE OF CERVICAL CYTOLOGY IN CERVICAL HPV INFECTION, Brian E. Ward, Barbara J. Burkett, Christine M. Peterson, and Christopher P. Crum, Departments of Pathology, Microbiology, Ob/Gyn, and Student Health, University of Virginia Health Sciences Center, Charlottesville, VA 22908.
A fundamental question in Papanicolaou smear screening is the specificity of cytological criteria for the recognition of HPV infection. To address this problem, we collected 532 cervical specimens from student women. Each cellular sample was pooled and portions were analyzed by Papanicolaou smear and molecular hybridization for HPV DNA. Twenty-eight specimens(5.3%) were HPV+ and 33(6.2%) contained cytological features either atypical or diagnostic for condyloma/CIN. In the latter group 13 contained prominently enlarged nuclei with or without cytoplasmic halos, and 8 (61.5%) were HPV+. Of the remaining 20 which contained less prominent cytological changes, two (10%) were HPV+. Our findings confirm: 1) that when sampling errors are minimized, HPV DNA can still be detected in approximately 5% of cytologically negative samples; 2) that certain cytological features correlate strongly with the presence of HPV; 3) that there exists a range of cytological abnormalities which do not correlate well with HPV+. This implies that the use of less stringent cytological criteria will not efficiently identify HPV+ cases.

I 163 MULTIPLE LOCI OF STATIC DNA BENDING IN PAPILLOMAVIRUS GENOMES, Van G. Wilson, Department of Medical Microbiology and Immunology, College of Medicine, Texas A&M University, College Station, TX 77843.

Loci of static DNA bending have been identified previously in conjunction with cis-active regulatory sequences in both prokaryotes and eukaryotes. To determine if similar static bends are present in papillomavirus genomes, DNA from BPV1, DPV, HPV1, HPV6, and HPV16 was screened by two-dimensional polyacrylamide gel electrophoresis. Multiple bend sites were detected in each genome, and the BPV1 genome was examined in detail. Two of the static bends in BPV1 DNA occur within the upstream regulatory region (URR). The location of each bend site was determined by electrophoretic analysis of circularly permuted constructs from the URR. The bend centers map to approximately nucleotides 7477 and 7790 respectively on the BPV1 genome. These bends occur at the 5' and 3' boundaries of a known DNase I hypersensitive site in the BPV1 minichromosome. In addition, the bend at 7790 is located at the 3' end of the E2 protein-dependent enhancer element from nucleotides 7611 to 7806. The potential role of the bend elements in the formation of nuclease hypersensitive regions and in transcriptional control is under investigation.

I 164 HPV IN CERVICAL CARCINOMAS: INCIDENCE AND SEQUENCE ANALYSIS USING POLYMERASE CHAIN REACTION D. K. Wright, H. Fox*, and M. M. Manos, Department of Infectious Diseases, Cetus Corporation, Emeryville, CA 94608; *Department of Pathology, UCSF, San Francisco, CA 94143.

Previous reports indicate that HPV DNA can be detected, by Southern blot analysis, in the majority of cervical carcinomas. We have used a more sensitive method, based on polymerase chain reaction (PCR) amplification, to investigate the incidence of HPVs in cervical carcinomas. Sections from fixed, paraffin-embedded cervical carcinomas were prepared for PCR and analyzed as follows: a) consensus primers, based on regions of DNA sequence homology between HPVs, were used to amplify regions of the viral genome; b) a consensus probe was used to detect the HPV DNA; c) amplification products were typed by hybridization with type-specific oligonucleotide probes. We used both the previously described system for amplification and typing in the L1 coding region (Manos et. al., Cancer Cells 7, in press) and an additional scheme for amplification and typing in the E6/7 coding region. The use of the E6/7 system ensures that viral sequences can be amplified from any samples in which L1 has been lost during viral DNA integration. We have also compared the DNA sequences of the HPV16 and HPV18 E6/7 PCR products from carcinomas and other sources to assess the degree of isolate-to-isolate variation within viral types.

I 165 DETERMINATION OF HPV DNA IN CERVICAL SAMPLES BY *IN SITU* HYBRIDIZATION H.-L. Yang, Y.Lee and L. J. Li ENZO Biochem, Inc., N.Y., N.Y. 10013

We have developed a non-radioactive *in situ* DNA hybridization assay for detecting HPV DNA in exfoliated cervical cells. Cervical samples were obtained either by scraping or by lavage. Specimens were transported and stored in transport medium at room temperature. Prior to hybridization, the cervical cells were briefly washed, then smeared onto slides and fixed. Hybridization was performed directly on the microscope slides using medium stringency hybridization conditions and biotinylated DNA probes specific for HPV types 6/11, 16, 18, 31, and 51. Detection was accomplished using a streptavidin-alkaline phosphatase detection system. The entire assay can be performed in about 2 hours.

The rapid colorimetric *in situ* hybridization assays used in these studies provide clear, readily detectable signals, low background staining, and excellent retention of cervical cell morphology. Our preliminary data indicates that the results of these *in situ* assays compare favorably with those obtained by Southern blot analysis of these same cervical samples.

I 166 THE PRESENCE AND EXPRESSION OF HUMAN PAPILLOMAVIRUS DNA IN ORAL SQUAMOUS CELL CARCINOMA. W.A. Yeudall[a], D.G. MacDonald[b], R. Smith[c], D.S. Soutar[c] and M.S. Campo[a]. a. Beatson Institute for Cancer Research, b. Glasgow Dental Hospital and c. Canniesburn Hospital, Glasgow, UK.

Nineteen tumour samples from patients with intraoral squamous cell carcinoma were screened for the presence of human papillomavirus (HPV) DNA by Southern blot hybridisation. Three of these (15.8%) were found to be positive, one each containing HPV-4, HPV-16 and HPV-18. Adjacent normal mucosa contained no or undetectable amounts of viral sequences. Normal buccal mucosa from 25 cancer-free individuals did not contain HPV DNA. In addition, all three viral genomes were expressed in the respective lesions, including the late genes, as detected by dot blot hybridisation. This is the first report of HPV-4 associated with an intraoral malignancy, and also the first report of expression of papillomavirus sequences in oral tumours.

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I 200 CONTINUED EXPRESSION OF HPV-16 E7 PROTEIN IS REQUIRED FOR MAINTENANCE OF THE TRANSFORMED PHENOTYPE OF CELLS COTRANSFORMED BY HPV-16 PLUS EJ-ras. Lawrence Banks[1], Jay Morgenstern, Lionel Crawford and Tim Crook, Molecular Virology Laboratory, Imperial Cancer Research Fund, Lincoln's Inn Fields, London, WC2A 3PX, [1]Ludwig Institute of Cancer Research, St. Mary's Hospital, London, U.K.

Recent studies have shown that the HPV-16 E7 gene encodes the major transforming activity of the virus in BRK cell transformation assays. To investigate the requirement for continued E7 expression in BRK cells transformed by HPV-16 E7 plus EJ-ras, we have developed a system for the inducible expression of the E7 gene. HPV-16 E7 under the control of the MMTV-LTR was transfected along with EJ-ras into primary BRK cells. In the presence of dexamethasone numerous transformed foci were observed although in the absence of hormone no transformed foci were obtained. A number of these foci were then picked and their dependence for growth upon continued E7 expression determined. In the absence of hormone E7 protein expression was not detectable. Upon addition of hormone E7 protein was observed within 2h. In comparison EJ-ras expression was unaffected. Maintenance of these cells in hormone free medium for periods longer than 48h resulted in complete cessation of growth, and after 10 days the cells did not resume growth upon readdition of hormone. These results show an absolute requirement for continued E7 expression for maintenance of the transformed phenotype in cells which have been transformed by HPV-16 E7 plus EJ-ras. Present studies are now aimed at elucidating the mechanisms by which E7 exerts this transforming activity.

I 201 PAPILLOMAVIRUSES E6 AND E7 ARE ZINC-BINDING PROTEINS

Miguel S. Barbosa, Douglas R. Lowy, and John T. Schiller. Laboratory of Cellular Oncology, National Cancer Institute, Bethesda, MD 20892 USA

The E6 and E7 proteins of all papillomaviruses have characteristic cys-x-x-cys repeats. Similar motifs have been shown to mediate metal binding in other proteins. We developed a modification of a published assay that detects radioactive zinc binding to proteins immobilized on filters and examined the zinc binding activity of papillomavirus E6 and E7 proteins. Using well characterized metalloproteins, we have determined that,under reducing conditions, this assay distinguishes proteins that coordinate zinc through cysteine residues from those that bind the metal through other amino acids. Under these conditions, bacterially synthesized E6 and E7 polypeptides of HPV18 and BPV1 exhibited high affinity zinc binding. The specificity for zinc was determined by blocking experiments in which the zinc binding was performed in the presence of increasing amounts of unlabeled zinc or other divalent metal ions. Given the presence of zinc ions and reducing conditions in mammalian cells, our results suggest that E6 and E7 are metalloproteins and may coordinate the metal ions through cysteine residues.

I 202 CONSTITUTIVE EXPRESSION OF HUMAN PAPILLOMAVIRUS TYPE 18(HPV18) EARLY GENES DRIVEN BY HUMAN CYTOMEGALOVIRUS (HCMV) EARLY PROMOTER RESULTS IN TUMORIGENIC CONVERSION OF NONTUMORIGENIC HeLa x FIBROBLASTS HUMAN CELL HYBRIDS, Dusan Bartsch, Elisabeth Schwarz and Harald zur Hausen, Angewandte Tumorvirologie, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 506, 6900 Heidelberg, FRG
In order to study the role of HPV18 gene expression for the malignant growth of human cells, nontumorigenic HeLa x human fibroblast hybrid cells (444) were transfected with a transcription unit (HCMV 7/23) consisting of HPV18 early E6 and E7 coding sequences (7/23) fused to human cytomegalovirus (HCMV) early promoter and enhancer element. All 10 tested cell clones (444(HCMV-7/23)) expressing both endogenous (driven by HPV18 noncoding region) and nonrearranged exogenous (driven by HCMV control element) HPV18 early genes are tumorigenic upon s.c. inoculation into nude mice. The tumor growth correlates with the level of exogenous HPV18 transcription. This strongly suggests a relationship between HPV18 early gene expression from the constitutive HCMV promoter and tumorigenic growth of previously nontumorigenic hybrid cells. No reduction of endogenous HPV18 E6-E7 gene transcription in tumors formed by 444(HCMV-7/23) cells was observed. The possible mechanisms of malignant conversion of the hybrid cells and the role of cellular interfering factors in this conversion will be discussed.

I 203 PRODUCTION OF TRANSGENIC MICE WITH HUMAN PAPILLOMAVIRUS SEQUENCES, Kong-Bung Choo and Lip-Nyin Liew, Recombinant DNA Laboratory, Department of Medical Research, Veterans General Hospital, Taipei, Taiwan 11217, Republic of China.
Human papillomavirus types 16 and 18 are closely associated with carcinomas of the anogenital tract, in particular, carcinoma of the cervix, and with epidermoid carcinomas of oral cavity. We have produced 8 transgenic mouse lines carrying the entire HPV16 or HPV18 genome, or the LCR/E6/E7 region of HPV16 which is thought to be involved in the transformation process. Physical analysis indicates that one of the lines carries three transgenic chromosomes with tandem duplications which have now segregated into three different lines. Another line also carries multiple integrations but probably in the same chromosome. Two of the lines are mosaics while other cases carry single integrations with tandem repeats. The HPV transgenes are stably inherited in the progenies in the cases analysed. Results on the analysis of the HPV gene expression will be presented.

I 204 SOLUBLE SUPPRESSOR FACTORS PRODUCED BY PERIPHERAL BLOOD MONONUCLEAR CELLS DURING PAPILLOMA VIRUS INFECTIONS, V. Chopra, S. Tyring and S. Baron, Department of Microbiology, University of Texas Medical Branch, Galveston, TX 77550. Patients with condyloma acuminatum (CA) and epidermodysplasia verruciformis (EV) have depressed cell mediated immunity, partially explained by an increased proportion of T cells with suppressor phenotype. Both are caused by human papilloma viruses resulting in progressive tumors of skin (EV) and occasional genital tract cancers (CA). Peripheral blood mononuclear cells (PBMC) from CA and EV patients when cultured for 3-5 days, released a soluble factor into the media that suppressed the phytohaemagglutin (PHA) driven proliferation and IL-2 production by lymphocytes from healthy controls. This soluble suppressor factor (SSF) was present in unusually high concentrations in culture supernatants from EV and CA patients but not from healthy controls. This SSF in various dilutions also prevented the proliferation of CTLL-2 cells in the presence of natural IL-2, measured by ^{3}H-thymidine uptake. The SSF had a molecular weight greater than 14,000, was heat labile, and acid labile at pH 2.0. Similarly, a soluble suppressor factor was released in the supernatants of PBMC from New Zealand white rabbits infected with Shope papilloma virus. This suppressor factor was also capable of aborting the *in vitro* IL-2 production and proliferation of normal rabbit PBMC in response to PHA and the *in vitro* proliferation of CTLL-2 cells in an IL-2 dependent assay. This inhibitor of IL-2 production/activity may block the T cell function and thus contribute to depressed cellular immunity in papilloma virus infections. This suppressor factor is being further characterized.

I 205 ANALYSIS OF HUMAN PAPILLOMAVIRUS TYPE 11 (HPV-11)-SPECIFIC mRNA SYNTHESIS IN HPV-INFECTED HUMAN UTERINE CERVIX *IN VIVO*, Karen D. Cockley,* Eilene Dolan,‡ William Hendrickson,* John W. Kreider*† and Mary K. Howett,* Departments of Microbiology and Immunology,* Pathology,† and Physiology,‡ The Pennsylvania State University College of Medicine, Hershey, PA 17033. A human xenograft model system in athymic nude mice permits replication of HPV-11 *in vivo* and results in morphologic transformation of virus-infected human grafts. The replicative events involved in HPV macromolecular synthesis in human tissue implants after HPV infection will be analyzed by *in situ* hybridization. Asymmetric RNA probes, prepared from HPV-11 cloned in the pGEM-1 vector, previously demonstrated abundant levels of HPV-11 transcripts and DNA in transformed human tissue grafts. A series of HPV-11-specific synthetic oligonucleotides (23-29 mers), corresponding to sense and anti-sense sequences from exon portions of each HPV-11 gene, were synthesized and purified. The specificity of [γ-^{32}P]ATP oligonucleotide probes was determined by DNA blot hybridization to digests of purified HPV-11 *Bam*HI-cleaved insert DNA. Specific synthetic oligonucleotides were 3' end-labeled with [α-^{35}S]dATP and used as probes to detect individual mRNAs or small subsets of the HPV-11-specific mRNA population after HPV infection at a variety of times by *in situ* hybridization and/or RNA blot hybridization. Experiments will be presented detailing synthesis of individual HPV genes during replication of HPV and during condylomatous transformation of human tissues.

I 206 CLONING AND TRANSCRIPTIONAL ANALYSIS OF HPV-11 CONTAINING A MUTANT E6 OPEN READING FRAME, Lex M. Cowsert and Carl Baker, Laboratory of Tumor Virus Biology, National Cancer Institute, Bethesda, MD 20892

A mutant HPV-11 genome was cloned from a liver metastasis of a primary lung carcinoma from a patient with juvenile laryngeal papillomatosis. HPV-11 had been previously detected in the benign, malignant and metastatic tissues (NEJM 317:873-878, 1987). Sequence analysis of the cloned HPV-11 genome revealed a duplication of the region between nucleotides (nt) 5936 and 277. As a result of this duplication two long control regions (LCR) are present in this virus. A consequence of this duplication is the creation of a mutant E6 open reading frame (ORF) under the control of one LCR and a wild type E6 ORF under the control of a second LCR. The mutant E6 ORF is comprised of nt 102 to 277 of the E6 ORF fused out of frame to the L1 region at nt 5936 with a translational termination codon at nt 5985. Polymerase chain reaction (PCR) has been used to determine the distribution of the wild type and mutant HPV-11 genomes in the benign, malignant and metastatic tissues. In addition PCR was used to demonstrate the presence of both wild type and mutant E6 transcripts in RNA extracted from the liver metastasis.

I 207 DETECTION OF E-4 ENCODED PROTEINS IN HPV-INFECTED TISSUE WITH ANTISERA TO E-4 SYNTHETIC PEPTIDES. Theresa Cromeans, Cosette Wheeler *, Joseph Icenogle, and Carlos Lopez. Viral Exanthems and Herpesvirus Branch, Centers for Disease Control, Atlanta, Georgia 30333 and *University of New Mexico, Albuquerque, New Mexico 87131

Although type specific epitopes of HPV-11 and 16 E-4 encoded proteins have been demonstrated (Brown, et al., 1988), the function of the E-4 encoded proteins is not yet known. E-4 proteins have been detected in HPV-1, HPV-2 and HPV-16 infected tissues, and most recently in HPV-11 infected transplanted tissue. Synthetic peptides of 16 amino acids corresponding to the complete amino acid sequences of an HPV-11 E1^E4 spliced protein were made. Human tissue infected with HPV-11 was implanted under the renal capsule of nude mice as described by Kreider et al. (J. Virol. 59:369, 1986). After 4 months cysts were removed and condylomatous cyst walls were fixed in formalin and paraffin embedded. Tissue sections showed the expected staining by an immunoenzymatic technique with antibody to papillomavirus common antigenic determinants (Dako). These tissues were also examined by immunoenzymatic staining for presence of epitopes reacting with rabbit antisera to the E-4 synthetic peptides. Intensive staining with antisera was obtained in the suprabasal layer where koilocytotic changes were apparent. In preliminary studies the pattern of staining was different from that obtained with the antisera to common antigenic determinants. This data as well as information on reaction of these antisera with human biopsy tissue infected with HPV-11 will be presented.

I 208 ACTIVATION OF INTEGRATED HPV16 E6/E7 GENES BY CELLULAR FLANKING SEQUENCES, Jun-yi Le, Gene Pecoraro and Vittorio Defendi, Department of Pathology, New York University Medical Center, New York, NY 10016.
We have reported (J. Virol., 62, 4420, 1988) that a 4.4kb genomic DNA fragment from a human anaplastic lung carcinoma, comprising part of the HPV16 authentic promoter, E6/E7 and part of E1 open reading frame and of cellular sequences is competent in transformation of NIH 3T3 cells; transformation competency resides in the whole hybrid molecule, since neither the separate viral and cellular sequences were active. We now report that this hybrid DNA fragment is also able to immortalize human cervical cells. Plasmid constructs with the chloramphenicol acetyltransferase (CAT) gene have been used to explore the function of the cellular sequences in gene regulation. The cellular sequences are not active in enhancerless transcription constructs, yet there is a clear enhancement of the reference gene, when they are placed downstream of the polyadenylation site in pSV_2 CAT. Enhancement by these sequences is orientation independent, however, it is location specific, i.e. downstream of the polyadenylation site, being not active when inserted at 5' of the promoter or at 3' of the pSV_2 CAT non-coding region. Deletion studies localize the activity in a fragment of 500bp, which contains a stretch of nucleotides having high homology with part of the U3 LTR region of certain human endogenous retroviruses.

I 209 ALTERNATIVE HPV16 EARLY MESSAGE TERMINATION SITES, J. Doniger, C. D. Woodworth, and J. A. DiPaolo, Laboratory of Biology, NCI, Bethesda, MD 20892. The predominant HPV16 mRNA species in 6 of 7 human cervical cell lines immortalized by recombinant HPV16 DNA was 1.8 kb, spanned bases 97-226, 409-880, and 3357-4234, and contained E6*, E7, E1/E4, and E5. But, in HCX16-5 it was 1.4 kb; a relatively low level of 1.8 kb was also detected. Moreover, other prominent species in HCX16-5 were similarly shorter. HCX16-5 was also the only line that contained a single HPV16 DNA integration site and no intact 7.9 kbp *Bam* HI fragment. The integrated HPV16 fragment spanned the 3'-end of L1, the long control region, the entire early region, and the 5'-end of L2. Further analysis of the mRNA with subgenomic HPV16 probes suggested that 1.4 kb species in HCX16-5 is a mixture of two truncated forms of the 1.8 kb species; either the E6 or E5 region was missing. A cDNA library of line HCX16-5 was constructed to examine more precisely the structure of its viral mRNA. 26 HPV16 specific clones were isolated from $\approx 10^5$ cDNAs. Analysis with subgenomic probes indicated that 6 were both missing E6 and truncated in the E5 region; of the remaining 20, 13 were truncated in E5, 4 were missing E6, and three contained both. Sequence analysis of the 5'-end of several clones with E6 show they begin between bases 110-119, just downstream of p97, the E6 promoter. One clone that is missing E6 begins in E7, 132 bases upstream of the E1 splice donor site at 880. The poly(A)-tail in one clone with E5 begins at 4234 just downstream from the poly(A)-signal at 4314, the standard termination region of the early messages. The poly(A)-tail of the clones truncated for E5 begins at 3820, 30 bases upstream from the end of E2. These data suggest that HPV16 contains a promotor in E7. Furthermore, the lack of complete L1 or L2 sequences changes the predominant termination site of the early messages.

I 210 GENETIC AND BIOCHEMICAL DIFFERENCES BETWEEN E6 AND E7 PROTEIN OF ONCOGENIC AND NON-ONCOGENIC TYPES OF HUMAN PAPILLOMAVIRUSES, Julia R. Gage and Felix O. Wettstein, Department of Microbiology and Immunology, UCLA School of Medicine, Los Angeles, CA 90024
Some genital human papillomavirus types have been implicated in the development of genital cancer. In addition, where adequate samples have been available, viral transcripts have been detected and mapping of these transcripts in cancers directly or in cancer-derived cell lines has shown that they can code for the E6 and predominantly for the E7 protein. These findings suggest that these two proteins play an important role in the progression to or maintenance of the malignant state. The overall goal is to see if there are differences in the properties between E6 and E7 of cancer-associated (oncogenic) genital papillomavirus types and E6 and E7 of the non-oncogenic types.

The investigations will include both genetic and biochemical approaches. The ability of oncogenic and non-oncogenic viruses to "transform" (alter morphologic or growth properties) epithelial cell lines or primary cells will be tested employing cotransfection with the selectable neomycin marker. The role of individual genes (proteins) will be investigated by inactivation or expression under heterologous promoters. Furthermore, the ability of subgenomic segments from oncogenic and non-oncogenic types to complement each other will be investigated.

For the biochemical analysis of proteins, they will be expressed first under strong heterologous promoters, such as the SV40 late promoter, to investigate protein modification (phosphorylation, glycosylation or acetylation), subcellular distribution and association with other proteins. Functional tests may involve assays for transactivation. E7 of HPV type 16 exhibits such properties.

I 211 ZINC-BINDING ACTIVITY OF HPV-18 E6 PROTEIN, Steven R. Grossman and Laimonis A. Laimins, Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL 60637

ZINC-BINDING ACTIVITY OF HPV-18 E6 PROTEIN, Steven R. Grossman and Laimonis A. Laimins, Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL 60637

We have expressed the E6 protein of HPV-18 at high levels in insect cells with a baculovirus vector. The predicted amino acid sequence of E6 contains repeating cysteine doublet motifs suggestive of a zinc-finger protein. E6 protein obtained from cells infected with a recombinant E6-expressing baculovirus is localized to membrane and nuclear matrix fractions and binds to DNA non-specifically. We have used a zinc-blotting technique to demonstrate that HPV-18 E6 protein also binds zinc. The binding is effectively competed only by divalent cadmium or cobalt but not by divalent magnesium or calcium, suggesting that the protein-metal interaction is occurring by coordination rather than by non-specific ionic interactions. Work is in progress to more accurately map zinc-binding protein domains using proteolytic fragments.

I 212 HUMAN PAPILLOMAVIRUS TYPE 6b AND TYPE 16 EARLY GENE EXPRESSION IN PRIMARY EPITHELIAL CELLS IN VITRO, Christine Halbert, Rebecca Blanton and Denise A. Galloway. Fred Hutchinson Cancer Res., Ctr., Seattle, Washington. 98104.

The transcription and replication of human papillomavirus DNA is linked tightly to the state of differentiation of the epithelial cell. To assess the role of epithelial cell differentiation in HPV gene expression, we have used retroviral vectors, packaging cell lines and infection of primary foreskin epithelium (HFE) with recombinant viruses. HPV 6b DNA from position 7454 to position 4421 was inserted into a retroviral vector (obtained from A.D. Miller) downstream of the neomycin drug resistance gene which is linked to the retroviral LTR. Expression of HPV 6b from its transcriptional regulatory region is being analyzed in submerged and air-liquid interphase cultures of epithelial cells grown in medium containing reagents affecting epithelial cell growth and differentiation.

We are also investigating the biological effect of HPV 6b and HPV 16 E6 and E7 ORF expression in primary human foreskin epithelium and in murine fibroblasts, 208F. For this purpose we have inserted the E6 and E7 ORFs either directly downstream of the retroviral LTR or using constructions containing the papillomaviral NCR. Infection of primary and established cell lines has been done with recombinant viruses. We are currently assessing the biological effect of these genes (E6 and E7).

I 213 PRIMARY SEQUENCE REQUIREMENTS WITHIN THE HYDROPHOBIC REGION OF THE BPV1 E5 PROTEIN. Bruce H. Horwitz, Debra L. Weinstat, Chris Leptak, and Daniel DiMaio, Department of Human Genetics, Yale University School of Medicine, New Haven, CT 06510.

The E5 gene of BPV1 is the primary transforming gene of the full length viral DNA in the C127 cell focus forming assay. The E5 gene encodes a 44 amino acid hydrophobic protein which has been isolated as a dimer from the membrane fraction of BPV1 transformed cells. Analysis of amino acid substitution mutations within the E5 protein suggests that there is a requirement for hydrophobic amino acids in the middle third of the protein. This result is consistent with the model that this portion of the protein mediates membrane association. We have now examined the specificity of the primary sequence requirements within the hydrophobic region of the protein by replacing these regions with random hydrophobic amino acids. Results of transfection assays indicate that in some cases random hydrophobic sequences can substitute for the first and second third of the E5 protein, implying that when linked to random hydrophobic sequences the carboxyl-terminal 15 amino acids of E5 can transform cells independently of most wild type E5 sequences. To confirm that wild type and mutant E5 proteins can transform cells idependently of other BPV1 sequences, we are developing an E5 expressing retroviral vector. Preliminary evidence suggests that retroviruses containing wild type E5 sequences are able to transform a large proportion of cells in culture implying that the recombinant retrovirus expresses the E5 gene at high levels.

I 214 ONCOGENE EXPRESSION IN BPV4-ASSOCIATED LESIONS *IN VIVO* AND *IN VITRO* Rhys T. Jagger, J. Gaukroger[1], M. Saveria Campo and W.H.J. Jarrett[1], Beatson Institute for Cancer Research and [1] Dept. of Vet. Path., Garscube Estate, Switchback Road, Glasgow G61 1BD UK

Cancer of the Upper Alimentary Canal in cattle in the Western Highlands of Scotland is associated with the synergistic action of Bovine Papillomavirus Type 4 (BPV4) and the ingestion of bracken fern (Jarrett *et al* Nature **274**, 215 (1978)). Radiolabelled EGF binding studies (Smith *et al.* Cancer Cells **5**, 267 (1987)) indicated a possible role for increased EGF receptor expression in this process. Analysis of primary bovine cell lines transformed *in vitro* by BPV2 indicates an increase in EGF receptor mRNA levels correlating with increased EGF binding to the cell surface. We have screened papilloma and carcinoma DNAs and cells transformed *in vitro* and have as yet found no evidence for EGF receptor gene amplification.

I 215 MALIGNANT TRANSFORMATION OF NIH 3T3 CELLS BY HPV 6 DNA. Mary S. Kasher[1], James A. McAteer[2] and Ann Roman[1]. Depts. of Microbiology and Immunology[1] and Anatomy[2], Indiana Univ. Sch. of Med., Indianapolis, IN. 46223

We have previously reported the morphological transformation of NIH3T3 cells by HPV6-T70 DNA, an HPV6b genome cloned from an invasive squamous cell carcinoma of the vulva. To further define the role of HPV6 in oncogenesis, we have analyzed three HPV6-T70-transformed NIH3T3 cell lines. Southern analysis indicated that the HPV6 DNA was present in an integrated form at <0.1 copy per cell suggesting that either the viral genome is maintained in a subpopulation of cells, a small segment of the viral sequences is maintained in all cells, or the viral genome acts via a "hit and run" transformation mechanism. All cell lines induced tumors when injected subcutaneously in nude mice. Histological analysis indicated tumor morphology characteristic of a highly cellular, spindle cell neoplasm. Tumor cell lines were established: cells were spindle-shaped, highly refractile, and grew in a highly disorganized manner, overgrowing each other at low cell densities. In contrast, the parental transformed cell lines displayed loss of contact inhibition only after reaching confluency. Southern analysis of tumor DNA is in progress, to determine if selection of a subpopulation of the parental transformed cells has occurred. In collaboration with M. Stoler, T. Broker, and L. Chow (Univ. of Rochester), we are analyzing both the transformed cell lines and the tumor cell lines for expression of viral mRNA via <u>in situ</u> hybridization, employing HPV6 exon-specific RNA probes.

I 216 HPV 16 AND 18 TRANSFORMED HUMAN EPITHELIAL CELLS, Pritinder Kaur and James K. McDougall, Fred Hutchinson Cancer Res. Ctr., Seattle, WA. 98104.

Primary human keratinocytes have been co-transfected with the dominant selectable marker gene pSV2neo and cloned HPV sequences. Selection in 50 ug/ml G418 yielded on average 13 colonies/ug neo DNA. Cell lines containing integrated primary HPV DNA have been established from a number of distinct epithelial cultures. These lines have been maintained <u>in vitro</u> for 1-2 years and have been characterized in terms of their growth requirements; keratin gene expression; differentiation capacity in response to confluence and high calcium; and tumorigenicity. Viral DNA, RNA and protein content will be discussed in the context of the biological phenotype of these cell lines.

I 217 INDUCTION OF THE BPV-1 MAJOR LATE PROMOTER IN VIRUS-INFECTED BOVINE FIBROBLASTS,
T.Stanley Burnett, Udo Kiessling* and Ulf Pettersson, Department of Medical Genetics, Biomedical Centre, Box 589, S-751 23 Uppsala, Sweden; and *Central Institute of Molecular Biology, Academy of Sciences of the GDR, Robert Rossle Strasse 10, 1115 Berlin Buch, German Democratic Republic.
Cycloheximide treatment of BPV-1 infected bovine conjunctival fibroblasts resulted in the synthesis of two major cytoplasmic polyadenylated RNA species which hybridized to a probe specific for the viral upstream regulatory region (URR). In contrast, these transcripts were not synthesized in virus infected murine C127 cells following cycloheximide treatment; instead a different pattern of URR-derived transcripts was observed (Burnett et al., Nucleic Acids Research 15, 8607-8620 (1987)). To investigate a possible difference in promoter activity within the URR, primer extension analysis was performed. A prominent 5´RNA initiation site was mapped to the viral major late promoter, PL, in cycloheximide treated infected bovine cells. The second initiation site in this region of the URR (P7185) was approximately four-fold less active than PL following cycloheximide treatment of the bovine cells, but was the dominant cycloheximide-induced URR 5´initiation site in infected murine C127 cells. Despite the apparently high level of induction of PL in the bovine cultures, only very low levels of polyadenylated RNAs containing late region sequences were detected, and synthesis of viral capsid antigen was not observed following the release of the cycloheximide block. These findings indicate that PL is not absolutely dependent upon keratinocyte-specific factors for activation.

I 218 PLURALITY OF HPV DNA TYPES IN AN STD POPULATION;
Andria Langenberg(1), James McDougall(2), Lawrence Corey(1), Richard Cone(1), from: (1)Dept. of Laboratory Medicine, University of Washington, Seattle, WA and (2)Fred Hutchinson Cancer Research Center, Seattle, WA.
Southern hybridization was used to determine HPV types in 94 genital specimens from 65 patients (50 women, 15 men) with genital warts. Overall, 65% (61/94) of the biopsies were positive for HPV DNA. The prevalence of HPV types were; HPV 6, 49%; HPV 11, 20%; HPV 16, 18%; HPV 18, 2%; HPV's 6+16, 3%; HPV 6+18, 3%, and; unrecognized HPV type, 2%. HPV positive blots were detected in 32% of specimens digested in ViraPap solution versus 65% positivity in specimens digested with SDS/proteinase K. Nine HPV DNA positive patients were sampled twice from the same site (6 vulvar, 1 cervical, 2 penile) over an interval of 2 weeks to 10 months. Of these 9, 5 had different HPV types in the first and second biopsies: 11+18; 6+16; 6+11; 11+16; 6+16. One patient had HPV 16 in both biopsies, and 3 went from positive in the first biopsy (HPV's 16, 6, and 6) to negative in the followup biopsy. Our results suggest that DNA typing of a single specimen in this population may not be adequate to prospectively assess HPV type.

I 219 INVESTIGATION OF E2 FUNCTION IN THE TRANSFORMATION OF PRIMARY CELLS.
Emma M. Lees and Lionel V. Crawford, Molecular Virology Laboratory, Imperial Cancer Research Fund, Lincoln's Inn Fields, London, WC2A 3PX.
It has been shown by this laboratory that DNA derived from the early region of HPV-16 will cooperate with the activated H-ras oncogene to transform primary baby rat kidney cells (BRKs) in the presence of dexamethasone. This communication describes the investigation of E2 function in this transformation process.
The E2 gene and deletion mutants thereof, have been cloned behind a strong heterologous promoter. These constructs were transfected into BRK cells together with activated ras, and HPV-16. We found that the presence of a highly expressing E2 construct overrode the requirement for dexamethasone in the transformation of BRK cells. A mutant containing just the transactivating domain of E2 gave similar results. Transformed colonies obtained in the presence of either dexamethasone or high levels of E2 were expanded. A series of immunoprecipitations were done using antibodies against HPV-16 E6 (kindly provided by Dr. D. Lowy and Dr. E. Androphy) and HPV-16 E7 (kindly provided by Dr. F. Wettstein) to investigate the levels of early protein expression in the cell lines. The levels of E6 and E7 seemed comparable in all cell lines analysed. The ability of these lines to cause tumours in syngeneic rats is now being studied. These results show that the transactivating ability of E2 is sufficient to lead to transformation of BRK cells by HPV-16 in association with ras. This finding may be important in the understanding of the role E2 plays in the transformation process in vivo.

I 220 EXPRESSION OF H-2 ANTIGENS IN CELL LINES OF C57BL/6 MOUSE ORIGIN TRANSFORMED WITH BPV 1. R.A. Mäntyjärvi, A. Laatikainen, H. Jägerroos, and H. Karjalainen, Dept. Clin. Microbiol. Univ. Kuopio, SF-70210 Kuopio, Finland. We have established a series of transformed cell lines and cloned sublines from primary mouse fibroblast cultures transfected with BPV 1 DNA. Several of these cell lines are tumorigenic, not only in nude mouse but also in immunocompetent syngeneic C57Bl/6 mouse. Immunization of syngeneic mice with the cell lines followed by in vitro stimulation was used to produce effector cells for cytotoxicity experiments. In these experiments, cell-mediated immune reactions of different specificities were observed. One of the factors regulating cell-mediated immune reactions is the level of expression of MHC molecules. We have tested a panel of our cell lines for H-2 antigens by two techniques. Monoclonal antisera were used in immunofluorescent staining of H-2K^b and H-2D^b antigens for a flow cytometer analysis. Alloreactive effector cells were generated in one-way mixed lymphocyte cultures of B57Bl and Balb/c spleen cells. Cytotoxicity of the effectors against the transformed cell lines was measured in an 18h ^{51}Cr release assay. The results so far have revealed a wide variation in the expression of H-2 antigens in the transformed cells. A more detailed analysis in comparison to the tumorigenicity is underway.

I 221 CYTOGENETIC ANALYSIS OF HPV-IMMORTALIZED HUMAN KERATINOCYTE CELL LINES, James K. McDougall, Pritinder Kaur, Eileen M. Bryant and Patricia P. Smith, Fred Hutchinson Cancer Research Center, Seattle, WA. 98104.

Cytogenetic analysis was performed on HPV immortalized human keratinocyte cell lines. Lines were established from individual clones after transfection of primary cells with HPV DNA. A number of lines containing integrated HPV18 or HPV16 DNA were analyzed.

All lines examined showed karyotypic abnormalities. Five of six lines were triploid with one near diploid cell line. Abnormalities included duplication of many chromosomes and various rearrangements, most often involving chromosome #1, #3 and #7. Marker chromosomes were present in all lines. Addition of TPA or subsequent transfection with an HSV-2 transforming sequence led to increased aneuploidy and additional marker chromosomes. Examination of one HPV18 containing cell line at 9, 23 and 62 passages in culture revealed that the line remained essentially stable over long periods in culture, retaining a near triploid chromosome number and several distinct marker chromosomes.

I 222 CORRELATIONS OF HPV INFECTION WITH THE EXPRESSION OF CELLULAR MARKERS OF GROWTH AND DIFFERENTIATION IN CERVICAL EPITHELIUM AND CULTURED CELLS. Ronald C. McGlennen, Ronald S. Ostrow, Linda S. Carson, Micheal S. Stanley and Anthony J. Faras. Institute of Human Genetics, Minneapolis, MN 55455. Human papillomavirus DNA is detected in the majority of cases of cervical dysplasias, and is thought to have a critical but insufficient role in the development of squamous cell carcinoma. The progression of HPV infected tissues to the malignant phenotype requires a promotional event involving mechanisms that regulate cellular proteins significant in cell growth and differentiation. Our preliminary studies on the HPV infected cell lines HeLa, SiHa and CaSki, derived from malignant tumors of the cervix, utilize flow cytometry to demonstrate the modulation of the cell surface receptors for transferrin (TFR) and epidermal growth factor (EGF) throughout the phases of the cell cycle as well as the expression of the differentiation antigens cytokeratin (CK), fillagrin (FIL) and involucrin (INV). Our data expands these concepts to include the study of cervical cells from patients with normal and dysplastic lesions. The molecular analysis of these tissues for HPV DNA by Southern blot will be correlated with the flow cytometric immunohistochemical measurement of TFR, EGF, CK, FIL, INV, and DNA ploidy to access the change in expression of these markers of growth and differentiation in a spectrum of premalignant clinical lesions.

I 223 VACCINATION AGAINST PAPILLOMAVIRUS-INDUCED TUMORS USING VACCINIA RECOMBINANTS EXPRESSING NON-STRUCTURAL PROTEINS. Guerrino Meneguzzi, U273 INSERM, Centre de Biochimie, Parc Valrose, 06034 Nice Cédex; François Cuzin, U273 INSERM, Centre de Biochimie, Parc Valrose, 06034 Nice Cédex; Marie-Paule Kieny, Transgène, S.A., 11 rue de Molsheim, 67000 Strasbourg; Richard Lathe, LGME-CNRS & U184 INSERM, 11, rue Humann 67000 Strasbourg; FRANCE.

Infection with papillomaviruses is associated with several types of cancers. Because infection with such viruses can be widespread we have sought to vaccinate against virus-carrying cells rather than against structural components of the virus. We previously reported that inoculation with live vaccinia virus recombinants expressing early proteins of polyomavirus can prevent development of polyoma-induced tumors and, additionally, can elicit tumor rejection in tumor-bearing animals. We have subsequently studied the properties of vaccinia recombinants expressing papillomavirus early proteins. The use of viruses expressing bovine papillomavirus (BPV1) antigents to vaccinate against BPV-tumors will be presented and the extension of this work to a human papillomavirus vaccine will be discussed.

I 224 GENETIC ANALYSIS OF THE COTTONTAIL RABBIT PAPILLOMAVIRUS (CRPV), Meyers, C. and F.O. Wettstein, Department of Microbiology and Immunology, School of Medicine, Molecular Biology Institute, and Jonsson Comprehensive Cancer Center, University of California, Los Angeles, CA 90024-1747. The cottontail rabbit papillomavirus (CRPV) is strictly epitheliotropic and transformation of fibroblasts *in vitro* has not readily been accomplished. To provide a realistic system for a genetic analysis, we developed an *in vitro* transformation assay using the rabbit skin epithelial cell line, sf1Ep. sf1Ep cells cotransfected with CRPV DNA and the G418 selectable marker developed resistant colonies which upon expansion exhibit an altered "transformed" morphology and form small colonies in soft agar. Tests for tumorgenicity in nude mice are in progress. Mutations in the ORFs E6, E7, E5 and E1 have been made and are being tested for their ability to transform sf1Ep cells. The mutants are also being tested directly in domestic rabbits for their ability to induce tumors. CRPV's E6 ORF codes for two proteins, a long E6 protein using the first AUG codon and a short E6 protein using the second AUG codon. Three E6 mutants have been constructed; of the two deletion mutants, one affects both E6 encoded proteins and one affects the long E6 protein only. A third mutant was made using site-specific mutagenesis to change the second E6 AUG codon to AUA. We have also shown that CRPV DNA with deletions in the late region but not wild type DNA induces foci efficiently in NIH 3T3 cells. Analysis of the DNA of rare foci induced by wild type DNA showed that spontaneous mutations had deleted part of the late region. We have cloned late region sequences into the late region of BPV-1 to test their effect on BPV-1's transforming of fibroblasts.

I 225 INCREASED TPA EXPRESSION IN C127 CELLS THAT HAVE BEEN SEQUENTIALLY TRANSFECTED WITH TWO DIFFERENT BPV BASED EXPRESSION VECTORS, Martin Montoya-Zavala, Hetty Leim, Michael Vrabel and Halina Sasak, Mammalian Expression Group, Integrated Genetics, Inc., Framingham, MA 01701.

Two different BPV-1 based vectors were constructed to express human tPA in murine cells. The first construct placed the tPA cDNA under the control of the metallothionein promoter and used the SV40 late poly A addition signal. Infection with this vector, followed by a number of subcloning steps, gave rise to a cell line called IIID2. The second construction had the same basic configuration to which was added a neo resistance marker under control of the metallothionien promoter and the SV40 early poly A signal. The neo-containing BPV-1 vector was used to super transfect the IIID2 cell line already expressing tPA. The retransfection of IIID2 (in the presence of selection with G418) increased the expression levels of tPA as much as four fold. The increase in protein level was correlated with an increase in mRNA but could not be attributed to increase of BPV vector copies. The increase of expression was not dependent on the presence of G418.

I 226 INTRAUTERINE PAPILLOMAVIRUS INFECTION IN HORSES, J. Moreno-López A. Eriksson, O. Nilsson, J.E.Rigmar and U. Pettersson. Department of Veterinary Microbiology and Department of Pathology, Faculty of Veterinary Medicine and Department of Medical Genetics, Biomedical Center Box 585, S-751 23 UPPSALA, SWEDEN. Papillomaviruses are widespread among mammals and the infection generally occurs on skin and mucous membranes. The papillomaviruses induce tumors in their natural hosts and rarely cross the species barrier. The spreading of virus between animals is thought to occur from one animal to another by direct or indirect contact. However, very little is known about the mecanisms of a vertical infection. We will present a case report of a foal born with a cutaneous fibropapilloma. The results of a histological study on the neoplasia and the characterization of the virus and its genome will be presented.

I 227 **CHANGES IN CELLULAR PROTEIN PROCESSING OBSERVED IN BPV-1 TRANSFORMED C127 CELLS APPEAR TO BE NOVEL EFFECTS OF AN E2-C GENE PRODUCT.** M. Kerry O'Banion and Donald A. Young. Departments of Medicine and Biochemistry, University of Rochester Medical Center, Rochester, NY 14642. Giant two-dimensional gel electrophoresis of proteins from cells transfected with whole or subgenomic portions of BPV-1 reveal a consistent set of six changes of which five appear specific for BPV-1. One of these changes [pvp 1; ~110 kd, pI 5.2] is an enhanced glycosylation of what we believe to be heat-shock/glucose-regulated protein 100 based on its position on our gels. Experiments to confirm this identity are in progress. Analyses of proteolytic cleavage products and pulse-chase experiments reveal that three of the other BPV-1 specific changes [pvp 2,3,4; 55-58 kd, pI 4.8] represent a single processed protein. However, at present it is unclear whether these changes arise from differential processing of a protein already present in C127 cells or represent a new protein product induced by BPV-1.

Several lines of evidence suggest that a portion of the E2 ORF, specifically the carboxy terminal region, is responsible for these unique changes in cellular proteins: 1) neomycin-selected C59-3881 cells harboring the full E2 ORF behind an SV40 promoter demonstrate these changes; 2) mutations in E4 and E5 ORFs have no effect on the protein phenotype*; 3) two mutations affecting the amino terminal portion of E2 have no effect*; and 4) an MSV promoted E2-C shows the changes. We hypothesize that in addition to its known ability to bind BPV DNA, E2-C may exert effects through interactions with cellular factors or DNA. Studies aimed at elucidating the mechanism(s) by which E2-C affects cellular proteins will be discussed. [Supported by grants from NIH (CA47650, DK16177), the Council for Tobacco Research USA (1774) and the Wilmont Foundation.]

Cell lines were generously provided by D. DiMaio, T. Haugen and L. Turek, and P. Howley.
*Levenson *et al.*, submitted.

I 228 CORRELATION OF PATHOGENESIS WITH VARIATIONS IN RESPONSE AND VIRUS PRODUCTION IN EXPERIMENTAL BOVINE PAPILLOMATOSIS, Carl Olson Department of Veterinary Science, University of Wisconsin, Madison, 53706 The development and duration of papillomas in various species vary considerably in individual animals. Little is known about the production of virus at different stages of papilloma growth. The bovine papillomatosis systems can provide much needed basic information on this naturally occurring disease in an out-bred population similar to man.

Two hundred forty fibropapillomas were produced on eight calves and observations made during more than three years. In some calves, the papilloma growth was progressive and rapid, in others slow and regressive. Frequent biopsies during the first three months provided materials for correlation of histological features, rate of growth and degree of virus production.

The significance of these observations in pathogenesis and course of papillomatosis will be discussed.

I 229 THE PHYSICAL STATE, EXPRESSION AND SEXUAL TRANSMISSIBILITY OF AN ONCOGENIC PAPILLOMAVIRUS IN RHESUS MONKEYS, Ronald S. Ostrow, Ann S. Viksnins, Bruce E. Kloster, M. Kathleen Shaver, Ronald C. McGlennen, Jay Tichelaar, and Anthony J. Faras, Institute of Human Genetics, University of Minnesota, Minneapolis, MN 55455. In a primate animal model system which closely mimics the natural disease associated with oncogenic human papillomaviruses of the genital tract, we have characterized papillomavirus DNA in tumors of Rhesus monkeys. Characterization of RhPV 1 DNA by restriction endonuclease mapping and nucleotide sequence analysis has revealed the nature of integrated RhPV 1 DNA and flanking cellular DNA. These data will be presented along with a comparison with various human PV integrated sequences and cellular DNA data banks. In addition, we will present the results of an analysis of metastatic tumor RNA for viral cellular run-on transcripts using RNA protection and PCR studies. (Such studies bear upon the possibility that integrated papillomaviruses may activate flanking cellular genes due to enhanced gene expression.) Finally, the results of a study for RhPV 1 infection of sexual cohorts of our index male Rhesus monkey will be detailed. This animal had a primary penile carcinoma as well as a metastatic lymph node tumor associated with RhPV 1 as determined by DNA hybridization analysis. The results show that latent RhPV infections occurred in a significant proportion of the sexual partners.

I 230 TRANSCRIPTIONAL TRANSACTIVATION OF AN SV40/HPV 1 TRANSFORMED HUMAN KERATINOCYTE CELL LINE USING AN SV40/BPV 1 E2 RECOMBINANT VIRUS, Adrian Parton, Paul Biggs, Daniel DiMaio*, Roger Grand, Phillip Gallimore, Department of Cancer Studies, The Medical School, University of Birmingham, B15 2TJ, England. *(current address) Department of Human Genetics, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06510.

The site of integration of HPV 1 DNA into an SV40/HPV 1 transformed human keratinocyte cell line (SVD2 (cyst)sp), has been determined by the in situ hybridisation technique to be at 2q32. Northern blotting analysis of this cell line identified HPV 1 specific transcripts representing approximately 0.01 % of the total mRNA population. However when this cell line was exposed to a recombinant SV40/BPV 1 E2 virus a concomitant increase in HPV 1 specific RNA's was observed, approximately 10 fold greater than the basal level of transcription in the parental cell line. Further Northern blotting analysis of cytoplasmic poly A+ RNA recovered from recombinant infected SVD2 (cyst)sp cells revealed a family of HPV 1 specific RNA's, the sizes of which ranged from 0.6 to 5.0 kb. HPV 1 E4 transcripts were detected by Northern blotting analysis and their splice junctions have been characterised using the polymerase chain reaction (PCR). Further investigation of the transactivated HPV 1 transcripts by cDNA cloning is currently in progress.

I 231 CHARACTERIZATION OF HPV16 and HPV18 IMMORTALIZED HUMAN CERVICAL CELL LINES. Gene Pecoraro, Michael Lee, Don Morgan and Vittorio Defendi, Department of Pathology, New York University Medical Center, New York, NY 10016.
Recent evidence has suggested a differential association of HPV16 and HPV18 with cervical carcinoma subtypes. We have reported (PNAS, in press) that HPV16 and HPV18 immortalize primary human cervical epithelial cells derived from the transformation zone of cervix uteri. Preliminary examination of our established cell lines in modified organotypical culture suggested that both viral types conferred invasive properties on the immortalized cells as well as alterations in differentiation [squamous (HPV16) vs. glandular (HPV18)] resembling the different histological subtypes of cervical carcinoma primarily associated with the different HPV types in vivo. In order to investigate these parameters (differentiation and invasiveness) two in vitro systems have been utilized, a multicell spheroid model and a further modification of the organotypical culture assay. Morphological and histochemical analysis of four HPV18 established cell lines examined thus far has revealed a glandular differentiation pattern with cytological features of anaplasia and the production of intracellular and extracellular mucin. The one HPV16 established cell line examined thus far clearly exhibits a pattern of squamous differentiation. Studies using monoclonal Abs specific for simple epithelia and stratified epithelia have revealed alterations in cytokeratin expression following immortalization by the two HPV types. Assays to elucidate the biochemical basis of the invasive properties of the cell lines are in progress; morphological and histochemical characterization of additional HPV16 and HPV18 immortalized cervical cell lines will be presented.

I 232 IN VITRO TRANSFORMATION OF NIH3T3 CELLS BY A MONKEY PAPILLOMAVIRUS (CGPV1), M. E. Reichmann, Alfred A. Reszka, John P. Sundberg, Department of Microbiology, University of Illinois, Urbana, IL 61801

A monkey papillomavirus (CgPV1), originally isolated from a penile lesion on a Colobus monkey, is being used as a model system for studies of human papillomavirus associated neoplastic diseases. The CgPV1 viral DNA exhibits similarities with the human papillomaviruses (HPV's) type 16 and 18, frequently found in cervical cancers. DNA similarities are reflected in terms of Southern blot hybridizations at high stringency (Tm-22°C) and nucleotide sequence homologies (up to 69%). In vitro transformation assays demonstrated a high transforming efficiency comparable to that of BPV1 DNA in NIH3T3 cells. The state of the CgPV1 viral DNA in these transformed cells is integrated and partially deleted, not unlike the genomes of HPV16 and HPV18 characterized in cell lines derived from cervical cells. Transcription analysis by Northern blot assays demonstrated no transcriptional activity in the E5 ORF region. The E6 and E7 ORF region is represented in all three transcripts evidenced in these analyses. The state of the viral DNA in transformed cell lines and the nature of mRNA species will be discussed.

I 233 DETECTION OF SPECIFIC VIRAL MESSAGES USING THE POLYMERASE CHAIN REACTION, M. Sanchez-Lanier, C. Lopez, J. Icenogle, M. Campion, Viral Exanthems and Herpes Virus Branch, Centers for Disease Control, and St. Josephs Hospital, Atlanta, GA 30333

Human papilloma viruses (HPV) types 16, 18, 31, 33, and 35 have all been implicated in cervical cancer. However, the diagnostic and prognostic significance of the various types is not well understood. Little is known about the mRNA expression within the various grades of HPV-associated lesions. We have used the polymerase chain reaction (PCR) to selectively identify viral messages in the HPV 16 carrying cell lines Caski and Siha, as well as tissue obtained from patients with cervical intraepithelial neoplasia and carcinoma in situ. The simplicity and sensitivity of PCR makes it a desirable technique for such analysis. RNA was isolated by the guanidinium method for total RNA preparation. Poly (A)+ RNA was obtained by two passages through an oligo(dt) cellulose column. The Poly (A)+ RNA was reverse transcribed using either poly $(dt)_{12-18}$ or specific primers. The PCR reaction was performed using primers which bridge known splice sites, as well as primers specific for known open reading frames. Contaminating DNA was controlled for by running the PCR reaction without an initial reverse transcriptase step. Results of mRNA analysis of Caski cells coincided with mRNA data previously published for this cell line. Specific viral messages found in cervical tissues will be presented.

I 234 MONOCLONAL ANTIBODIES RAISED AGAINST THE E7 ORF OF HPV18 - CROSS REACTIVITY WITH HUMAN CELLULAR COMPONENTS, L.A. Selvey, R.W. Tindle, J. Smith, I.H. Frazer, Lions Immunology Laboratory, Princess Alexandra Hospital, Brisbane, Queensland, Australia

23 monoclonal antibodies were raised against an HPV18 E7-MS2 replicase fusion protein[1] and were specific for the E7 portion of the fusion protein. They were all cloned twice by limiting dilution. Competition assays reveal a cluster of 5 epitopes. With the exception of one antibody, which cross reacts with E6 of HPV18, the antibodies do not cross react with other HPV MS2 replicase fusion proteins[1] (E618, E118. E716, E616, E416 & L11216) in ELISA assays. All of the antibodies recognize an appropriate 27kd band when reacted against the E718 fusion protein in Western Blot.

These antibodies have been reacted against the HPV18 containing cell lines C41 and HeLa in Western Blots. As well as a 12kd band which is the predicted molecular weight for the E7 ORF of HPV18, several of the antibodies also recognize bands of different molecular weights. The antibodies were also reacted in Western Blots with a variety of non HPV containing cell lines, both transformed and non transformed, and bands were produced with all but 5 of the antibodies. Different monoclonal antibodies produced bands of different molecular weights and bands were produced in non transformed cell lines. Immunofluorescence suggests that the cellular proteins recognized by the antibodies are cytoplasmic. This apparent cross reactivity of the HPV18 E7 monoclonal antibodies with cellular proteins is currently being characterized further by immunoprecipitation and further Western Blots. It is of interest to ascertain whether this cross reactivity represents a true similarity between viral and cellular proteins which may have functional or immunological significance for the virus as it interacts with human cells.

[1] Seedorf et al, The EMBO Journal, 1987, 6(1), 139.

I 235 GENETIC ANALYSIS OF THE TRANSFORMING POTENTIAL OF THE BOVINE PAPILLOMAVIRUS E5 GENE IN AN ACUTE ASSAY. Jeffrey Settleman, Amin Fazeli, Bruce Horwitz, and Daniel DiMaio. Department of Human Genetics, Yale University School of Medicine. New Haven, CT 06510.

We have constructed a BPV/SV40 recombinant virus which can be packaged in monkey cells to produce high titer virus stocks. Cells infected with the virus efficiently express the BPV E2 transactivating gene and the E5 transforming gene. Within 24-48 hours of infection, mouse C127 cells undergo a dramatic morphologic change and begin to overgrow the monolayer, appearing similar to cells stably transformed by BPV DNA. Genetic mapping experiments indicate that the E2 and E4 genes, which are also contained in the virus, are not required for this effect, whereas a mutation that disrupts the E5 gene is completely defective. Infection of quiescent, serum-starved cells with wild type virus, but not with an E5-deleted virus, results in the induction of cellular DNA synthesis, indicating that E5 expression allows cells to synthesize DNA in the absence of serum. We have cloned numerous E5 missense mutants into the recombinant virus and determined their activity in this acute transformation assay. It appears that the hydrophobic character of the middle third of the protein, as well as specific amino acids in the carboxyl terminus are required for transforming activity. Overall, there is a good correlation between the ability of mutants to stimulate DNA synthesis after infection and to give rise to stably transformed foci after transfection, suggesting that the ability of the E5 protein to allow cells to escape quiescence and undergo DNA synthesis may be an important role of the protein in inducing stable transformation.

I 236 EVIDENCE FOR COOPERATIVITY BETWEEN E2 BINDING SITES IN THE REGULATION OF BPV-1 TRANSCRIPTION, Barbara A. Spalholz and Peter M. Howley, Laboratory of Tumor Virus Biology, National Cancer Institute, Bethesda, MD 20892. The E2-dependent enhancer $E2RE_1$ contains two pairs of E2 binding sites separated by 114 nucleotides. Although a single pair of binding sites functions as an E2-dependent enhancer, the intact $E2RE_1$ activates transcription in the presence of E2 to a much higher level and has been shown to be required for the activation of BPV-1 promoters P_{89} and P_{7940}. We have mutated numerous sites within $E2RE_1$ and have assayed these mutations in the CAT expression vector pA10CAT. Although no positive acting elements could be found in the sequences between the E2 binding sites, this region can block enhancer activity if it is present between an intact pair of binding sites and the target promoter. The presence of a single E2 binding site between this inhibitory region and the promoter could however rescue the enhancer activity of that pair of E2 binding sites. This finding indicated that the E2 binding sites of $E2RE_1$ interact over some distance in a cooperative manner to regulate transcription. Similar interactions may also be involved in the regulation of other BPV-1 promoters. The P_{2443} promoter is also transactivated by the E2 gene product, but this activation requires the long control region (LCR), which contains $E2RE_1$, to be present in *cis*. Directly upstream of the putative TATA box for P_{2443} is a single E2 binding site. We have investigated the contribution of this site in E2 transactivation through mutations and have found that destruction of this E2 binding site does reduce the response of this promoter. This is evidence of cooperation between this E2 binding site and other E2 binding sites in the LCR to regulate transcription from P_{2443}.

I 237 PRODUCTION OF THE HPV 16 E7 GENE PRODUCT VIA A RECOMBINANT VACCINIA VIRUS AND CHARACTERISATION OF ANTIBODIES THAT REACT WITH THE EXPRESSED PROTEIN. Jane C. Sterling, Cornelia S. McLean, Anthony C. Minson, Department of Pathology, University of Cambridge, UK.

Polyclonal and monoclonal antibodies were generated against the HPV 16 E7 gene product expressed in a prokaryotic expression system. The sera of rabbits immunised with purified E7-beta galactosidase fusion protein were found to react in Western blot analysis with a 20kd protein in a lysate of cells infected with a vaccinia virus-HPV 16 E7 recombinant. By immunofluorescent and immunoperoxidase staining the E7 protein in the vaccinia recombinant infected cells was identified in the nuclei, in contrast to reports of its cytoplasmic localisation in CaSki and SiHa cell lines and in a cervical cancer. The protein was not detected by these antisera in cells infected with wild type vaccinia virus.

The fusion protein was then used as an immunogen to produce monoclonal antibodies and the resulting hybridoma supernatants were screened using the vaccinia recombinant as a target antigen. Three E7-specific monoclonal antibodies raised in this way detected a 20kd product in the vaccinia recombinant by immunoprecipitation and Western blotting and gave a strong nuclear signal in immunofluorescent and immunoperoxidase tests. A protein of the same molecular weight was immunoprecipitated from CaSki cells by these antibodies. The antibodies are now being used also used to screen for the localisation of E7 protein in tissues and cells containing HPV 16 sequences.

I 238 MUTATION OF THE ZINC FINGER OF HPV16 E7 DOES NOT ABOLISH CO-TRANSFORMATION WITH *ras*. Alan Storey, Neil Almond and Lionel Crawford, ICRF Tumour Virus Group, Cambridge University Department of Pathology, Tennis Court Road, Cambridge, CB2 1QP, U.K.

Recently it has been shown that the E7 gene of HPV types 16, 18, 31 and 33 can cooperate with *ras* to transform baby rat kidney (BRK) cells, and that the N-terminal part of the E7 gene has homology to conserved regions of adenovirus E1a, Zinc-finger motifs, first identified in the transcription factor TFIIIA, coordinate Zn^{++} and direct binding of the protein to DNA. Mutations within the C-terminal of E7 which abolish a putative Zn-finger structure decreases the ability of E7 to bind Zn^{++} and to cooperate with *ras* to transform BRK cells. The transformed cells have greatly altered morphology, are highly vacuolated and form tumours in immunocompetent rats. The Zn^{++}-finger of HPV16 E7 is much larger than that of TFIIIA and since E7 and E1a are unlikely to bind to DNA directly, the Zn^{++}-finger could be important for protein-protein interactions.

I 239 INHIBITORY EFFECT OF RETINOIDS ON PROMOTER-DEPENDENT AND -INDEPENDENT CELL TRANSFORMATION INDUCED BY BOVINE PAPILLOMAVIRUS DNA, Siu Sing Tsang, Erika Cheng, Paul Sobkin and Hans F. Stich, Environmental Carcinogenesis Unit, British Columbia Cancer Research Centre, Vancouver, B.C. V5Z 1L3, Canada

Mouse C3H/10T½ cells underwent cell transformation following transfection with bovine papillomavirus DNA (BPV DNA). The frequency of cell transformation is severalfold higher in the presence of tumour promoters (promoter-dependent transformation) than in the absence of tumour promoters (promoter-independent transformation). The effect of retinoids on BPV DNA-induced cell transformation was studied. Tumour promoters used included mezerein and teleocidin. Both retinoic acid and retinol inhibited promoter-dependent and -independent transformation, regardless of whether the retinoids were applied concurrently with the promoters or 7 days after the cells had been treated with promoters. Promoter-independent transformation was more sensitive to retinoid treatment than promoter-dependent transformation. In order to inhibit the frequency of promoter-independent or -dependent cell transformation by 70%, treatment with 10^{-7} and 10^{-6}M of retinoids was required, respectively. Our results suggest the role of tumour promoters in cancer development induced by papillomavirus, and support the usefulness of retinoids as chemopreventive agents for papillomavirus-related cancer.

(Research supported by grants from the Natural Sciences and Engineering Research Council of Canada and the National Cancer Institute of Canada).

I 240 **EXPRESSION OF HUMAN INTERLEUKIN-3 USING BOVINE PAPILLOMAVIRUS VECTORS**

Rob W. van Leen, Janny G. Bakhuis and Patty J. Lemson, Department of Heterologous Gene Expression - Research and Development, Royal Gist-brocades N.V., PO BOX 1 2600 MA Delft, The Netherlands

Interleukin-3 (IL-3), a multilineage colony stimulating factor (multi-CSF) supports the proliferation of human marrow hematopoietic progenitors to form granulocytes, erythrocytes, macrophages and megakaryotes. In order to reach this state of differentiation synergy with other factors such as GM-CSF, G-CSF, M-CSF and EPO is required. The biological and pharmaceutical properties of this important lymphokine can only be determined with extensively purified factor. It is virtually impossible to obtain sufficient amounts of IL-3 from natural sources. Hence, we have expressed the protein in several host organisms using r.DNA techniques. Human IL-3 expression vectors were constructed using the cDNA sequence from Dorssers *et al.* (Gene 55 (1987), 115-124). The cDNA was expressed from BPV-derived expression vectors using different promotors. Expression of the complete IL-3 gene was also analyzed using comparable constructs. Data will be presented to compare the effectivity of gene expression using the various constructs. The IL-3 synthesized in C127 cells is compared with bacterially expressed IL-3 with respect to post-translational modifications and biological effectivity.

I 241 MORPHONUCLEAR CELL IMAGE CHARACTERIZATION OF EPITHELIAL CERVIX CELLS INFECTED BY HPV 16 & 18 AS REVEALED BY MOLECULAR HYBRIDIZATION.
Marc Verstraete, Robert Kiss (*), Dominique d'Olne (+), Yvan de Launoit (*), Patrick Van Alphen, Robert Paridaens (§), Arsène Burny and Guy de Martynoff. Lab. Biological Chemistry, Dept. Molecular Biology, Fac. Sciences; (*) Lab. Histology, Fac. Medicine; (+) Dept. Anatomopathology and (§) Clin. Dépistage, Inst. J. Bordet; Free University of Brussels, Belgium.
We analyzed in double-blind the relationship existing between 15 morphonuclear parameters and the level of HPV 16-18 infection, as assessed by DNA hybridization. We used the SAMBA 200 cell image processor, with software allowing discrimination of morphometrical (nuclear area), densitometric (DNA quality and quantity) as well as textural (chromatin organization) parameters monitored on 27 Feulgen-stained human cervical smears. Our results indicate that all the 15 morphonuclear parameters evolve in a continuous manner from "level 1" (non-infected cells) to "level 3" (highly infected cells), the "level 2" representing borderline cases, i.e. suspect smears that seemed weakly infected as revealed by low 32P- (viral HPV 16 and 18) and high 3H- (human "ALU" sequence) labeled DNA hybridization. Furthermore, only 3 out of the 27 cases appeared as "infected" following human pathological examination whereas 8 out of the 27 cases corresponded to "level 2 infection" and 8 to "level 3 infection", as independently assessed by molecular as well as cell image analyses.
Supported by IRSIA, FRSM and Lefevre Foundation, Belgium.

I 242 **HUMAN KERATINOCYTES (HKc) IMMORTALIZED BY HUMAN PAPILLOMAVIRUS (HPV) DNAs ASSOCIATED WITH CERVICAL CANCER EXHIBIT ABERRANT TERMINAL DIFFERENTIATION**, C. D. Woodworth, and J. A. DiPaolo, Laboratory of Biology, NCI, Bethesda, MD 20892. HKc immortalized by HPV types associated with cervical cancer were used to compare the morphologic and molecular alterations associated with expression of HPV genes. A technique was adapted that permitted *in vitro - in vivo* correlations by transplanting monolayers of HK, removed intact from culture dishes by digestion with dispase, beneath a dorsal skin-muscle flap of nude mice. Although normal HKc formed a well differentiated epithelium, HPV16 and 18-immortalized cells differentiated aberrantly demonstrating a series of changes that mimicked cervical intraepithelial neoplasia grades 1 to 3. No invasion of the adjacent connective tissue was observed. Similar results occurred *in vitro* as HPV-immortalized cell lines stratified poorly and contained a cell subpopulation resistant to differentiation promoted by serum or loss of anchorage. Steady state levels of RNAs expressed during normal cervical differentiation were either unchanged (involucrin, keratin 1) or slightly increased (laminin receptor). Thus, HKc that contain integrated and transcriptionally active HPV DNAs exhibit aberrant differentiation both *in vivo* and *in vitro*. Using this model it will be possible to identify the underlying causes associated with pathologic alterations characteristic of cervical intraepithelial neoplasia.

Late Additions

I 243 IMMORTALISATION OF KERATINOCYTES BY HPV16, McCance, D.J., and Price, T., Department of Microbiology, UMDS, Guy's Campus, Guy's Hospital, London SE1 9RT U.K.
We have shown that the HPV16 genome on its own can immortalise primary human foreskin keratinocytes and inhibit differentiation when the cells are grown on collagen gels on raft cultures (PNAS 85:7169). The three mRNA transcripts produced in these cells all code for the E6-E7 region of HPV16. We now show that HPV16 E6-E7 region alone is able to immortalise keratinocytes and inhibit differentiation. The complete HPV6 genome on its own failed to immortalise primary keratinocyte cultures.

I 244 DETECTION AND SEQUENCING OF HPV DNA AND RNA USING THE PCR, M.A. Johnson[1], I.S. Bevan[1], T. Bromidge[2], N.J. Maitland[2] & L.S. Young[1], [1]Dept. Cancer Studies, University of Birmingham, U.K., [2]Dept. Pathology, University of Bristol, U.K.

We have previously reported the detection of HPV types 11 and 16 in cervical smear cells using the PCR (Young et al., 1989, Brit. Med. J., in press). This work has been extended to investigate (a) HPV 16 transcription in cervical carcinoma, (b) the DNA sequence of both oral and cervical isolates of HPV 16 and (c) the presence of different HPV types in biopsies of cervical carcinoma.

Viral transcription over the E6-E7 region of HPV 16 has been analysed with the PCR using oligonucleotide primers spanning the major and minor splices on RNA isolated from the CaSki cell line. The predicted splices over this region have been confirmed using this technique which is currently being applied to the investigation of splice patterns over the HPV 16 E6-E7 region in fresh tumour biopsies.

The DNA sequence of the E6 region of HPV 16 isolates amplified by PCR has been analysed. Oral and cervical isolates derived from either normal or tumour specimens had identical DNA sequences over the E6 region. Thus, the HPV 16 E6 sequence appears to be invariable irrespective of tumour-association or tissue origin.

The PCR was used to detect HPV types 6, 11, 16 and 18 in paraffin-embedded biopsies of cervical carcinoma. HPV was detected in 16/19 cases, 6 of which contained all 4 subtypes. These results indicate a high level of multiple HPV infection in cervical cancer.

HPV Diseases and Therapy

I 300 TYPE-SPECIFIC COLPOSCOPIC MANIFESTATIONS OF GENITAL HPV INFECTION AMONG RANDOMLY SELECTED GYNECOLOGICAL OUTPATIENTS, Markku Aho, Pekka Nieminen, Valeria R. X. Soares, Ervo Vesterinen, Antti Vaheri, Jorma Paavonen, Department of Obstetrics and Gynecology, University Central Hospital and Department of Virology, University of Helsinki, Finland

Colposcopy has become increasingly important in the recognition of multifocal HPV infection. Our purpose was to study defined colposcopic manifestations of cervical, vaginal, and vulvar HPV infection among randomly selected gynecological outpatients. A dot blot technique was used for the detection of HPV types 6, 11, 16, 18, 31, 33, or 35 (Viratype, Life Technologies, Inc., Gaithesburg, MD). The study population consisted of 85 HPV DNA-positive cases (16 had HPV 6/11, 31 had HPV 16/18, 30 had HPV 31/33/35, and 8 had mixed HPV types) and 19 matched HPV DNA-negative controls enrolled in a prospective cohort study. 81% of the cases and 100% of the controls had satisfactory colposcopy. Compared to controls, cases were more likely to have an atypical transformation zone (ATZ) (61% vs. 32%), exophytic condylomas (8% vs. 0%), non-exophytic lesions of the vagina (47% vs. 16%), and of the vulva (76% vs. 37%). The colposcopic score (derived from the border features, color tone, and vascular atypia features of ATZ) was higher among cases than among controls (5.3 vs. 1.4). However, the score did not discriminate between different HPV DNA genotype groups. Among the cases, several individual colposcopic features showed type-specific associations: exophytic condylomas with HPV 6/11; cervical leukoplakia with HPV 16/18; cervical satellite lesions with HPV 6/11; focal white epithelium (WE) of the vagina with HPV 16/18; distinct WE of the vulva with HPV 16/18 and HPV 31/33/35; vulvar satellite lesions with HPV 16/18; vulvar filaments with HPV 6/11 and 31/33/35; and vulvar fissures with HPV 31/33/35. Thus, although we were unable to distinguish cervical ATZ features by HPV type, we were able to demonstrate type-specific associations with several other defined cervical, vaginal, and vulvar colposcopic patterns.

I 301 *Open clinical trial of the efficacy and safety of 0.5% Podofilox solution in the treatment of genital warts in the female. D.A. Baker, M.D., B. Patsner, M.D., J. Quigley, R.N., Department of Obstetrics and Gynecology, State University of New York, Stony Brook, New York, 11794.*

Podofilox (formerly Podophyllotoxin) (0.5% solution) is the most active compound of podophyllin resin. Purification and standardization of this substance as well as patients being able to apply the solution themselves provide additional benefits to the therapy of genital tract disease caused by condyloma. The study was an open clinical trial in female patients to test the safety and efficacy of this preparation. Patients with a clinical diagnosis of genital warts gave written consent. Inclusion: wart count ≤ 20 and a total area of $< 20\ cm^2$. In addition, patients enrolled did not have any prior treatment for the last month. Patients were treated with a twice daily application for three consecutive days followed by a four day drug free period. Patients were treated for a maximum of four treatment cycles and evaluated weekly for four weeks then on week six and ten.

A total of six patients were enrolled in the treatment protocols. Five of the six patients treated in this open protocol had excellent response to the Podofilox treatment. Four patients had complete erradication of their vulvar disease. At the end of four weeks, the percent of original wart count was reduced by 85% in these six patients.

No systemic adverse reactions were noted, however, moderate local reaction was noted in four out of six patients and one severe local reaction in one out of six patients. This preliminary study suggests that Podofilox is safe and effective in treating peritoneal warts in women.

I 302 HPV-DNA IN FOMITES USED FOR THE MANAGEMENT OF PATIENTS WITH GENITAL HPV INFECTIONS, Christine Bergeron, Alex Ferenczy and Ralph M. Richart, I.P.E.C.A., Paris, France; Departments of Pathology and Gynecology, The Sir Mortimer B. Davis Jewish General Hospital, Montreal, Quebec, Canada, and Columbia University College of Physicians and Surgeons, New York, New York. We have investigated the possibility that HPV could be present in cells adhering to objects that are used for the management of patients with genital HPV infections. HPV-DNA was identified by filter hybridization (ViraPap™/ViraType™, Life Technologies, Gaithersburg, MD) in swabs taken from eight of 16 (50%) surgical gloves; 23 of 62 (37%) and one of 62 (1.6%) biopsy forceps, before and after sterilization in 30% tinture of Savlon for 30 minutes, respectively. Twelve of 22 (54.5%) and one of 22 (4.5%) cryoprobe tips tested positive for HPV-DNA before and after cleaning with 90% ethanol solution for one minute, respectively. Whether HPV on fomites is infectious has not been evaluated in this study. However, based on the low rate of HPV-DNA positivity following sterilization, washing and soaking them in 2.5% formaldehyde disinfectant solution (Sonnacide) with viricidal property should be adequate. The same surgical gloves should not be used for external and internal examination of the lower anogenital tract.

I 303 A MULTICENTER DOUBLE-BLIND CLINICAL TRIAL OF THE SAFETY AND EFFICACY OF 0.5% PODOFILOX SOLUTION IN THE TREATMENT OF GENITAL WARTS, K. R. Beutner[1], M. A. Conant[1], A. Friedman-Kien[2], University of California at San Francisco, San Francisco, CA 94117[1], and New York University, New York, NY 10016[2]

Podophyllin resin has been used in the treatment of genital warts for the past forty years. Podofilox (formerly podophyllotoxin) is the most biologically active component of the resin. The present study was designed to evaluate the safety and efficacy of 0.5% podofilox solution applied by patients for the treatment of genital warts. One hundred and six male patients were enrolled and randomly allocated to active or placebo groups. The two groups were comparable in terms of age, sex, initial wart count, and wart area. Half of the patients had their warts for > 12 months. The test medications were applied by the patients in treatment cycles of twice daily application for three consecutive days followed by a four day rest period (no treatment). Patients were treated for a maximum of four treatment cycles and evaluated weekly during the treatment phase and at Week 6. At each visit wart count, area, physician assessment, and any local or systemic adverse reactions were noted. Based on physician's assessment 35/41 (92%) patients at the end of treatment (Week 4) in the podofilox group responded (> 50% improvement) while 3/28 (8%) responded in the placebo group ($p<0.001$). Twenty-seven of the 53 patients (51%) in the podofilox group completely healed during the treatment period. Sixteen of these 27 patients (59%) remained clear. Two of the 53 patients (4%) treated with placebo cleared. At the end of Week 4, the mean percentage of original wart count and area in the podofilox group were 26.4% and 17.7% respectively versus 91.7% and 95.8% in the placebo group ($p<0.001$). There were no systemic adverse reactions. Transient local reactions of pain, burning, erosions, and inflammation were noted during the treatment phase. Twenty-nine patients from both groups by Week 4 developed new warts remote from the initial treatment sites, indicating the active nature of HPV infection. This study demonstrates the safety and efficacy of 0.5% podofilox administered at home by patients for the topical treatment of genital warts.

I 304 THE PstI-XhoII RESTRICTION FRAGMENT OF THE HPV6b L1 ORF LACKS IMMUNOLOGIC TYPE-SPECIFICITY AS DETERMINED BY SERA FROM HPV6-INFECTED PATIENTS AND CONTROLS. William Bonnez, Robert C. Rose, Richard C. Reichman, and David G. Strike. Department of Medicine, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642. We have identified two regions within the L1 ORF of HPV6b which encode polypeptides that cross-react with BPV1 antisera. These regions are expressed from the XhoII-PstI fragment (fragment 480) and the PstI-XhoII fragment (fragment 594) (Strike, et al. J Gen Virol, in press). Using TrpE fusion proteins, Jenison et al (J. Virol 1988; 62:2115) indirectly found that the fragment 594-encoded polypeptide reacted with a majority of sera from patients with condyloma acuminatum but not with sera from 6-month old babies. Here we report experiments in which the E. coli produced TrpE fusion protein expressed directly from fragment 594 was tested in Western blot assays with sera from 36 patients with biopsy proven HPV6 disease and with sera from 22 nuns with no lifetime sexual activity. The Western blot procedure was done in a Miniblotter manifold (Immunetics) with an alkaline phosphatase conjugate detection system. Each serum sample was tested after preadsorption with Blotto buffer (1), lysate of E. coli containing TrpE protein alone (2), lysate of E. coli containing a TrpE fusion protein expressed from the 5' end of the HPV6b L2 ORF (3), and lysate of E. coli containing the TrpE fusion protein expressed from fragment 594 (4). A serum that reacted with TrpE-594 after presadsorption with (1), (2) and (3), but not after preadsorption with (4) was considered positive. Eighteen of 36 (50%) patients, and 8/22 (36%) nuns were positive. Seven of 14 (50%) of the nuns with a past or present history of nongenital cutaneous warts were seropositive. These results suggest that the polypeptide encoded by fragment 594 is not specific for the detection of HPV6 infection in adults. In addition, the study supports our previous observation that the polypeptide encoded by fragment 594 contains a cross-reactive antigenic region.

I 305 IN SITU HYBRIDIZATION TO HUMAN PAPILLOMAVIRUS DNA IN FIXED TISSUE SAMPLES: COMPARISON OF DETECTION METHODS, S. E. Bromley, M. M. Darfler, M. L. Hammer, A. Jones-Trower, M. A. Primus, J. W. Kreider*,+Molecular Diagnostics Division, Life Technologies, Inc., Gaithersburg, MD 20877 and *Milton S. Hershey Medical Center, Penn. State University, Hershey, PA 17033

In situ hybridization has been used to identify HPV-infected cells in fixed embedded tissue samples. The technique has been successfully applied to archival material in retrospective analysis, and to current samples for prospective analysis. While the results are unaffected by the age of properly fixed tissue (C. Wheeler, pers. comm.), some aspects of tissue preparation and hybridization, including probe synthesis, are critical. In addition, there are various detection schemes currently used to localize probe-target hybrids. We have compared autoradiographic, fluorescent and colorimetric detection methods to determine their relative sensitivities for in situ detection of HPV DNA. As an evaluation system, we have employed experimental condylomata produced by transplantation of HPV 11-infected human foreskin into athymic mice. The resulting cysts provide a large supply of uniform material, allowing side-by-side evaluation of a variety of detection strategies. The resulting cysts provide a large supply of uniform material, allowing side-by-side evaluation of a variety of detection strategies. The cysts are also more closely related to biopsy samples than the cancer cell lines commonly used as model systems for HPV-infected tissue. After optimizing the conditions for each detection technique, we performed hybridizations under probe-limiting conditions. By decreasing the probe concentration while the HPV 11 DNA target concentration remains constant in the cyst, we have compared the efficiency of several different detection schemes. We will present the results of this comparison, and discuss the merits of each technique employed.

I 306 POST-TREATMENT NONOXYNOL-9, Henry W. Buck, M.D., Head of Gynecology, Watkins Student Health Center, Univ. of Kansas, Lawrence, KS 66045.

HPV gains access to lower epithelial layers through surface breaks. After treatment with laser or cryotherapy, new lesions may develop in the midst of epithelial healing. Recurrences with all types of treatment have proven discouraging to clinicians and patients alike. "Normal" appearing epithelium may contain viral particles. Although not proven in vitro to be effective against HPV, Nonoxynol-9 has been shown to be lethal for HSV and HIV. In an effort to discourage growth of new non-cervical lesions following cryotherapy, patients were instructed to apply spermicide cream containing Nonoxynol-9 to treated and adjacent areas at bedtime.

Effectiveness was judged by comparing the numbers of patients lesion free (colposcopic visualization after application of 5% acetic acid) after 1-2 cryotherapy treatments with those requiring 3 or more, before and after institution of the spermicide regimen. In the female, the percentage with only 1-2 treatments increased from 75% to 88%. In the male with 5 or fewer lesions it increased from 79% to 96%; with more than 5 lesions, from 33% to 71%. These increases are significant by statistical analysis.

Reducing the viral load is the goal of HPV treatment; Nonoxynol-9 appears helpful. Spermicide cream is safe and usually well tolerated and with condoms is also recommended for intercourse after treatment and in new relationships. Nonoxynol-9 after cervical cryo or laser is being investigated.

I 307 CHARACTERISATION OF AN ANTIGENIC REGION OF L1-HPV-16 WHICH CONTAINS AN HPV-16 TYPE-SPECIFIC AND AN HPV-16/HPV-2 RESTRICTED EPITOPE.

J. Cason, D.Patel, J. Naylor, D. Lunny, P.S. Shepherd, J.M. Best & D.J. McCance. Laboratory of Cancer Virology, St Thomas Hospital and Departments of Immunology and Microbiology, Guys Hospital, U.M.D.S., London SE1 7EH, United Kingdom.

L1 proteins of papillomaviruses are highly conserved, making serological characterisation of HPVs difficult. We have made monoclonal antibodies (Mabs) to L1-HPV-16 (172-375) and investigated their epitopes by assessing reactivity to a nested set of synthetic peptides and, L1 proteins from other HPV types. One Mab recognised HPV-16, but not 6, 11, 18, or 2; three others recognised HPV-16 & 2 but not 6b, 11, or 18. All 4 Mabs bound to peptide 269-284: Mab HPV-16/HPV-2 cross-reactivity may be explained by recognition of PDDLYIK (HPV-16) and PDELYIK (HPV-2: i.e. a difference of a -CH2 group). The HPV-16 type-specific epitope probably includes a unique sequence at the -COOH region of 269-284. Peptide 269-284 was recognised by polyclonal antisera raised against L1-HPV-16 (172-375) but not by antisera to BPV-1. Thus, we have identified an immunogenic region on the L1-HPV-16 which contains an HPV-16 type-specific epitope.

I 308 TYPING OF HUMAN PAPILLOMAVIRUS FROM CARCINOMAS OF THE UTERINE CERVIX IN KOREA BY POLYMERASE CHAIN REACTION, Samhyun Cho[1,3], Magnus Evander[1], Eva Rylander[2], Doo Sang Kim[3], and Goran Wadell[1], [1]Dept. of Virology, Univ of Umea, [2] Dept. of Obstet. & Gynecol., University Hospital, S-901 85 Umea, Sweden, [3] Dept. of Obstet. & Gynecol, Hanyang Univ Sch of Med, Seoul 133, Korea

To relate possible presence of HPV DNA to different clinical stages of carcinoma of uterine cervix in Korea, two approaches were used. First, all specimens were analysed for the presence of HPV 6, 11, 16, 18, 31, and 33 DNA by Southern blot hybridization. Second, in order to evaluate the reliability of the Southern blot, cases negative in the Southern hybridization were analysed with the polymerase chain reaction (PCR) by using primer pairs specific for HPV 16,18, and 33 DNA. The prevalence of HPV DNA in uterine cervix carcinoma in Korea was 45% (18/40) by Southern hybridization. Southern negative specimens were found to be positive (20/22) by means of PCR. Therefore, the total prevalence rate was 95%(38/40). Furthermore, we found that Southern hybridization was less efficient for detection of HPV DNA in specimens from far advanced stages. Among specimens from stage IIIb to IV, only 2/9 were positive. The majority of HPV DNAs were type 16(33/40, 83%), and all 5 adenocarcinomas contained HPV 16 DNA. No difference in the presence of HPV DNA in cells of varying degree of differentiation was found. Chemoresponse was independent of whether the Southern analysis for HPV 16 DNA was positive or negative. Therefore, PCR must always be used to validate a negative result obtained by any other method.

I 309 IDENTIFICATION OF HUMAN PAPILLOMAVIRUS DNA ON ROUTINE PAP SMEARS, Young J. Choi Y, Nelson Yee, and Stanley Bauer, Department of Pathology, The Bronx-Lebanon Hospital Center, Bronx, NY 10456.

Human Papillomavirus (HPV) DNA usually has been identified by Southern blot assay or by in situ hybridization (ISH) using radiolabeled probes. These techniques are long, cumbersome and present radiohazards. To develope a rapid routine method of detecting HPV DNA in exfoliated cells from cervico-vaginal specimens, 550 cases of routine Pap smears were destained in acid alcohol for 30 min. and ISH was conducted on the smears using biotinylated HPV 6/11 and 16/18 DNA probes followed by counterstaining with EA 50. In the smears showing cytological changes of HPV infection or condyloma (koilocytosis or warty atypia), HPV 6/11 and/or 16/18 were detected in 45% of cases. In intraepithelial cervical neoplasm (CIN) with koilocytotic changes, HPV 16/18 was detected in 54% of cases. In 3% of negative Pap smears, HPV DNA was detected in normal appearing squamous cells. These results are comparable to those obtained by Southern blot assay or ISH using radiolabeled probes. Therefore, ISH using biotinylated probes on routine smears can detect the presence of the HPV genome and associated cytological changes at the single cell level. Since ISH using a biotinylated probe is a rapid, simple procedure, ISH in conjunction with a Pap smear may be used as a screening procedure to detect HPV infection.

I 310 IMMUNOLOGICAL CROSS-REACTIVITY TO EXPERIMENTALLY-PRODUCED HPV-11 OF POLYSERA RAISED AGAINST BACTERIALLY-EXPRESSED FUSION PROTEINS OF HPV-6 AND HPV-16 L1 AND L2 ORFS, Neil D. Christensen, John W. Kreider, Nancy M. Cladel and Denise A. Galloway, Departments of Pathology, Microbiology and Immunology, The Milton S. Hershey Medical Center, Hershey, PA 17033, and Fred Hutchinson Cancer Research Centre, Seattle, WA 98104 Polysera were raised in rabbits against bacterially-expressed fusion proteins of the L1 and L2 open reading frames (ORFs) of HPV-6 and HPV-16 (Cancer Cells [Cold Spring Harbor] 5:105, 1987). These four sera were tested for reactivity to laboratory-produced HPV-11, BPV-1 and CRPV in a series of immunological assays including: 1)immunoperoxidase staining of the koilocytotic nuclei in sections of formalin-fixed, paraffin-embedded HPV-11 experimental condylomas generated in the athymic nude mouse xenograft system (Nature 317:639, 1985); 2)immunoperoxidase staining of koilocytotic nuclei in fresh frozen sections of HPV-11 condylomas; 3)reactivity both to intact and disrupted HPV-11, BPV-1 and CRPV particles in ELISA; 4)reactivity to viral proteins of HPV-11, BPV-1 and CRPV in Western blots; and 5)neutralization assay for HPV-11 experimental condylomas. The four sera positively-stained koilocytotic nuclei in paraffin and fresh-frozen tissue sections of HPV-11 condylomas. In Western blots, the polysera raised to HPV-6 L1 fusion protein identified the major capsid protein (57 kda) of HPV-11, but did not react with either BPV-1 or CRPV proteins. In contrast, the sera raised against HPV-16 L1 also cross-reacted with the CRPV major capsid protein (60 kda), but not with BPV proteins. Staining in Westerns by sera raised against the L2 proteins did not identify any major bands despite positively-stained tissue sections. Reactivity to the papillomaviruses as determined by ELISA indicated that these sera reacted very poorly to intact viral particles, but strongly with alkaline-disrupted viral particles. HPV-11 neutralization assays were attempted also with these sera, and preliminary results indicated that no neutralization occurred. We conclude that: 1)the bacterially-expressed proteins of HPV-6 and HPV-16 did not induce in rabbits an antibody response to group-specific antigen; and 2)the antibodies generated against all four fusion proteins generated cross-reactivity predominantly to internal as opposed to external epitopes on HPV-11 capsid proteins.

I 311 LYMPHOPROLIFERATIVE RESPONSE TO HPV16 FUSION PROTEINS IN HPV16 POSITIVE PATIENTS,
Mary E. Connor, Anna G. Ghosh, John R. Arrand, Departments of Immunology and Molecular Biology, Paterson Institute for Cancer Research, Christie Hospital and Holt Radium Institute, Manchester, M20 9BX, UK.
HPV16 can be identified in 60% of cervical carcinomas and 20-40% of cervical intraepithelial neoplasias (CIN). However, not all patients with HPV16 infection develop a frank carcinoma, suggesting a difference in immune response. To assess this, HPV16 fusion proteins were used to determine the lymphoproliferative response of peripheral blood mononuclear cells (PBMCs) of patients with either CIN or cervical carcinoma. All were known to be HPV16 positive.
Fusion proteins containing the HPV16 open reading frames L1 and E6 were produced by expressing the respective open reading frames in a bacterial system. Patients with varying degrees of CIN were typed using the "ViraPap" detection kit on cervical smears and/or Southern blotting of colposcopically directed biopsies. A third group known to be HPV16 negative, with or without other current HPV infection eg. hand warts, were used as a control.
The lymphoproliferative response was assessed after 3, 5 and 7 days. Initial results suggest that the L1 protein is more antigenic than E6. No difference between the CIN and cancer patient groups has yet been detected, but more patients are to be analysed before further conclusions are drawn.

I 312 **DETECTION OF ANTIBODIES SPECIFIC TO HUMAN PAPILLOMAVIRUS TYPE 16 (HPV 16) IN HUMAN SERA : USE OF SYNTHETIC PEPTIDES AND TIME RESOLVED FLUORESCENCE**
Guy de Martynoff [1], Robert Brasseur [2], Philippe Vennin [3], Patrick Van Alphen [1], Arsène Burny [1] and Marc Zeicher [4]. [1] Lab. Biol. Chemistry ; [2] Macromol. at Interface, Free University of Brussels, Belgium; [3] Centre Oscar Lambret, Lille, France; [4] IRE-Celltarg, Fleurus, Belgium.
Selected on the basis of secondary structure (GARNIER) and hydrophilicity (EISENBERG), five potential immunogenic peptides - derived from E6, E7, L1 and L2 ORFs - were synthesized, ranging from 9 to 15 aminoacids. Coupled to BSA, these peptides were used in the very sensitive DELFIA procedure to study the humoral response to HPV 16. Specific immunoglobulins directed against these viral proteins were detected in human sera using biotinylated monoclonal anti-IgG antibodies, Europium-labelled streptavidin, and the Arcus Time Resolved Fluorometer (L.K.B. Wallac).
Cut off value (mean + 3 S.D.) was determined in a population putatively free of HPV 16 (children of 1 to 5 years old). While antibodies level above this cut off value could not be found in any children, significant response was observed in 7% of normal adults (blood bank donors) and in 33% of women with invasive cervical carcinoma. This latter result was well correlated with the presence of the HPV 16 genome in the tumor of the patients, as assessed by Southern blot.
With the sensitivity of this procedure higher than ELISA, the use of synthetic epitopes appears to be suitable for the detection of potentially oncogenic HPVs in serological testing of patients.

I 313 HPV GENOMES IN EXFOLIATED CELLS FROM THE MALE URETHRA,
G.Della Torre, R.Donghi, A.Longoni, S.Pilotti, M.A.Pierotti, G.De Palo and G. Della Porta, Istituto Nazionale Tumori, Milano, Italy.
Male sexual partners of women with HPV-associated genital lesions are at high risk for developing penile lesions and transmitting viruses. Since the incidence of HPV infection in males is far lower than in females, inapparent HPV infection should be found in male partners. In an attempt to determine whether urethral epithelial cells can be a hidden site for HPV infection, we used the Southern blot hybridization procedure to examine 56 urethral smears from partners of women with regressed or evident condylomata and/or intraepithelial neoplasias. Seventeen of the 56 DNA specimens yielded almost no detectable DNA. Viral DNA sequences were detected in 6 (15%) of the 39 available DNA samples. HPV 18, 6 and 11 were found in 4, 1 and 1 specimens, respectively. Only 2 of the 6 positive patients had penile condylomata. These preliminary data suggest that HPV can reside in the distal urethral tract and thus may be sexually transmitted.

I 314 DETECTION OF NUCLEAR ANTIGENS PARTLY ENCODED BY THE E2 ORF OF HPV TYPE 16 IN CIN AND CERVICAL CARCINOMA USING ANTIPEPTIDE ANTIBODIES. J. Dillner, L. Dillner, J. Robb, I. Jones, J. Willems, R. Lerner and R. S. Smith.
Dept. of Virology, Karolinska Institute, Stockholm, Sweden; Research Institute of Scripps Clinic and Johnson & Johnson, La Jolla, CA, USA.

A 20 residues synthetic peptide deduced from a region close to the carboxyterminus of the E2 ORF of HPV 16 was found to be reactive with IgA antibodies in 24 of 33 sera from patients with cervical intraepithelial neoplasia (CIN) or cervical carcinoma, whereas only with 6 of 27 sera from healthy donors. The peptide was used to generate monoclonal antipeptide antibodies and to immunoaffinity purify naturally occuring human antipeptide antibodies from carcinoma in situ patient sera. Both the mouse monoclonal and the human antipeptide antibodies detect a nuclear antigen in HPV-16-carrying CIN biopsies, in 2 cervical carcinoma cell lines (CaSki and C4II) and in HPV-16-transfected NIH3T3 cells, whereas not in HPV-negative cervical biopsies, in HPV-negative cervical carcinoma cells (C-33A and HT-3) or in untransfected NIH3T3 cells. By immunoblotting these antipeptide antibodies detect four E2-encoded antigens of molecular weights 26, 48, 51 and 58 kDa.

I 315 Prevalence of IgA antibodies to the papillomavirus capsid antigen in human sera. Lena Dillner and Joakim Dillner. Department of Virology, Karolinska Institute, S-10521 Stockholm, Sweden.

We previously reported the existence of IgA antibodies to the PV capsid antigen in cervical secretions as well as a correlation of IgA anti-PV in secretions to the diagnosis of cervical intraepithelial neoplasia (CIN) (Dillner et al, Int. J. Cancer, 1988). In order to investigate if the titers of IgA anti-PV in serum showed a similar disease-related distribution, 44 sera from patients with CIN or invasive cervical cancer in comparison with 16 sera from healthy donors were tested for IgA antibodies to purified bovine papillomavirus virions. Whereas about 60% of CIN grade 1 patients had IgA anti-BPV antibodies, only 4 of 16 (25%) of healthy donors had these IgA antibodies. Patients with carcinoma in situ or invasive cervical cancer only had these IgA antibodies in 5 of 15 cases, in line with the infrequent expression of PV capsid antigen in these lesions. In a separate study, we also investigated the IgA anti-BPV levels in a population of 90 sera from a normal population with regard to age and sex distribution. The IgA anti-BPV antibody titers were found to be high also in children under age 5 and in males.

I 316 LEUKOREGULIN UP-REGULATION OF NK AND LAK LYMPHOCYTE DESTRUCTION OF PAPILLOMA VIRUS (HPV) IMMORTALIZED HUMAN CERVICAL EPITHELIAL CELLS (HCX). Paulette M. Furbert-Harris, Charles H. Evans, Craig D. Woodworth and Joseph A. DiPaolo, Laboratory of Biology, National Cancer Institute, Bethesda, Maryland 20892.
HPV-immortalized HCX and HPV DNA-positive cervical carcinomas are being evaluated as a model of lymphokine modulation of epithelial cell sensitivity to natural immunologic cytotoxicity. Leukoregulin (LR), a 50 kd lymphokine, selectively increases the plasma membrane permeability and sensitivity of many tumor cells to NK and LAK cell killing. The presence of NK cells in cervical neoplasia and absence of specific T-cell reactivity in patients with HPV infection indicate the logic of this approach. Epithelial cells, immortalized by recombinant HPV16 DNA, were treated with 2.5 u/ml LR for 1hr and mixed with NK cells or IL-2 induced LAK cells at E:T ratios up to 50:1 in a 4 hr ^{51}Cr release assay. Both early and late passage HPV16 immortalized HCX were resistant to NK but sensitive to LAK cytotoxicity. LR induced modest sensitivity to NK ($P<0.05$) and markedly up-regulated LAK sensitivity 1.5 to 4-fold. QG-U and C4-1 cervical carcinoma cells with integrated HPV16 and -18 DNA, respectively, were resistant to NK but sensitive to LAK. The response of LR treated QG-U and C4-1 cells was similar to HPV16-immortalized HCX, i.e. LR conferred sensitivity to the NK resistant tumor cells and dramatically increased their sensitivity to LAK. Although HPV-immortalized HCX containing integrated HPV DNA are non-tumorigenic they mimic the response of HPV16 or 18 positive cervical carcinoma cells. These observations provide a foundation for the usefulness of this model in evaluating the therapeutic potential of leukoregulin alone, or in combination with other cytokines or chemotherapeutic drugs in the prevention and treatment of cervical dysplasia and neoplasia.

I 317 REARRANGEMENTS OF HPV 16 GENOME IN BENIGN AND MALIGNANT LESIONS OF THE VULVA, R. Donghi, G. Della Torre, A. Longoni, P.O. de Campos Lima, S. Pilotti, M.A. Pierotti and G. Della Porta, Istituto Nazionale Tumori, Milano, Italy. The genomic DNA from 26 biopsies of different vulvar lesions (6 condylomata, 1 Buschke-Loweinstein tumor, 2 VIN II, 5 VIN III, 1 verrucous carcinoma, 4 early carcinomas and 7 invasive carcinomas) was subjected to Southern blot hybridization with HPV 6, 11, 16 and 18 DNA probes. Fourteen of the 26 lesions (53%) were HPV positive and HPV 16 was detected in 10 lesions. Alterations of HPV 16 DNA restriction pattern, due to rearrangements and/or integration of the viral sequences in the cellular genome, were detected in 9 of the HPV 16 positive lesions, i.e. 1 VIN II, 4 VIN III, 3 early carcinomas and 1 invasive carcinoma. A preliminary analysis using subgenomic regions of HPV 16 indicated that a portion of the HPV 16 E1 open reading frame (ORF) was deleted in 1 of the early carcinomas. In addition, a region comprehensive of E6, E7 and part of the E1 ORFs was duplicated in the HPV 16 positive invasive carcinoma. Finally, a duplication of L1-L2 HPV 16 ORFs was detected in a flat condyloma, thus indicating that rearrangements of viral genome can be detected also in the early stages of the malignant trasformation.

I 318 EPIDEMIOLOGY OF GENITAL WARTS IN A SEXUALLY TRANSMITTED DISEASES CLINIC. JM Douglas, AJ Davidson, AC Cardozo, FN Judson. Denver Disease Control and Univ. of CO Health Sciences Center, Denver CO. To better define secular trends in rates of genital warts (GW) and the characteristics of patients with GW attending a sexually transmitted diseases (STD) clinic, we reviewed yearly statistics from 1975-1987 and a computerized database of 13,659 records of initial clinic visits for 1987 for the Denver Metro STD Clinic. From 1975-1987, numbers of visits for GW by men and women increased 470% and 410%, respectively, and by 1987, GW had surpassed gonorrhea (GC) in accounting for more clinic visits than any other STD.

Clinic Visits	1975	1977	1979	1981	1983	1985	1987
Men-GW	388	922	1245	1836	1832	1927	2224
GW/GC	0.19	0.34	0.40	0.55	0.76	0.86	1.92
Women-GW	147	258	216	414	328	342	749
GW/GC	0.14	0.23	0.21	0.49	0.48	0.45	1.96

In 1987, GW rates were higher in whites than hispanics and blacks (15.0% vs. 10.2% vs. 6.6%), in contrast to higher rates for GC in blacks than hispanics and whites (20.7% vs. 12.3% vs. 6.0%). Peak GW rates in women were in those $\leq$ 19 yrs old (12.9%), the group with the highest GC rates. Men $\leq$ 19 yrs old also had the highest rate of GC, but those 20-29 yrs old had the highest rates of GW (14.1%). Prior episodes of GW had occurred in 43% of GW patients and 12% of all clinic patients. While 92% of GW patients had symptoms of STD, only 66% noted genital or extragenital lesions, and among patients attending for asymptomatic screening, 3% had GW. GW are a problem of growing importance in STD clinics, appear to affect a population somewhat different than that with GC, and are often unrecognized by patients.

I 319 HUMAN PAPILLOMAVIRUS (HPV) DNA IN CO_2-LASER GENERATED PLUME OF SMOKE AND ITS CONSEQUENCES TO THE SURGEON, Alex Ferenczy, Christine Bergeron and Ralph M. Richart, Departments of Pathology and Gynecology, The Sir Mortimer B. Davis Jewish General Hospital, Montreal, Quebec, Canada; I.P.E.C.A., Paris, France, and Columbia University College of Physicians and Surgeons, New York, New York. CO_2 laser energy is absorbed by intracellular water but not by proteins or nucleic acids. The possibility of dispersing viral DNA during laser therapy of HPV-containing genital infections has been explored using a filter hybridization technique (ViraPap™/ViraType™, LTI, Gaithersburg, MD). Samples were taken using dacron swabs from 110 patients in nine separate treatment sessions as well as from five filters, four fume vacuum tubes (Stackhouse Point One System™) and the nasopharynx, eyelids and ears of the laser surgeon before and after laser surgery. The viral RNA probes were specific for groups of HPV types 6/11, 16/18 and 31/33/35. HPV-DNA was identified in swabs from 65 of 110 (60%) lesional tissues. One of the five filters tested in 37 (2.7%) HPV-DNA positive lesions contained HPV-DNA type 6. The four fume vacuum tubes tested from 28 HPV-DNA positive lesions were HPV-DNA negative as were the nasopharynx, eyelids and ears of the operator. Although HPV-DNA may be released during laser vaporization of genital HPV infections, providing appropriate equipment for evacuating HPV-DNA positive smoke is used, contamination of the operator is unlikely.

I 320 VERTICAL TRANSMISSION OF HUMAN PAPILLOMAVIRUSES: DETECTION BY DNA AMPLIFICATION, K.H. Fife, F. Bubalo, D.L. Boggs, and J. Gaebler, Departments of Medicine and Pediatrics, Indiana University School of Medicine, Indianapolis, IN.

We had previously shown that HPV DNA could occasionally be detected in the foreskin tissue of normal, healthy newborns. To better assess the frequency and risk factors for such transmission, we prospectively evaluated 234 pregnant women for HPV infection of the cervix and then evaluated foreskin tissue and oral cavity scrapings from the infants born to these women. While 26/234 women (11%) were positive for HPV DNA in the cervix by Southern blot, none of the specimens from the infants was positive by this method. To improve the sensitivity of detection, we used the polymerase chain reaction (PCR) DNA amplification technique employing the *Thermus aquaticus* DNA polymerase. We synthesized primers from the long control regions of HPV types 6, 16, and 18 and used these to retest the infants of HPV-positive mothers. The HPV 6 primers were also able to detect HPV 11 DNA. In 10 instances, specimens were available from both a mother who was known to have one of these 4 HPV types and her infant. The PCR assay was performed using 0.5 - 1 μg of cellular DNA which was amplified for 40 cycles. Known negative specimens were interspersed as a control for cross-contamination of specimens. Products of the PCR reaction were electrophoresed on agarose gels, transferred to nitrocellulose, and hybridized with ^{32}P-labeled, cloned HPV DNA of the corresponding type. Infants from 4 of these pairs were positive for HPV of the same type as the mother. One infant was positive for HPV 11 and 3 were positive for HPV 18. Two of the positive infants had both a foreskin and an oral cavity scraping available for testing and both sites were positive in both cases. We conclude that vertical transmission of HPV may occur relatively commonly. The clinical significance of such transmission is currently uncertain but requires further investigation. Highly sensitive detection systems such as PCR should permit more careful noninvasive follow-up of exposed infants.

I 321 HOST FACTORS WHICH PREDICT FAILURE TO RESPOND TO CONVENTIONAL ABLATIVE THERAPY FOR GENITAL HPV INFECTION, Ian H Frazer, Dan O'Connor, Lynn Kennedy, Pat Hogan, Lions Human Immunology Laboratories, Department of Medicine, University of Queensland, Princess Alexandra Hospital, Woolloongabba 4102.

Human genital papillomavirus infection frequently recurs after conventional ablative therapy. Clinically obvious immunosuppression, including HIV infection, is known to predispose to recurrence. Other factors associated with increased risk of recurrence are not well identified. We investigated clinical features associated with recurrence of genital HPV infection after ablative therapy, for 182 patients who had no clinically obvious immunodeficiency disease. 100 responders were compared with 82 non-responders. It was established that patients with recurrent disease were more likely to be smokers (54% versus 31%: $p< 0.01$), have a high serum IGE level (274 versus 128 kiu: $p< 0.01$), have no history of prior palmar or plantar warts (65% versus 86%, $p< 0.02$), and have evidence of past HSV2 infection (36% versus 24%: $p< 0.05$). The two groups were comparable for all standard measures of immune function (T cell counts, immunoglobin G, A and M levels, recall of delayed type hypersensitivity). We conclude that host factors other than immunocompetence may be important in determining the outcome of treatment for human genital papillomavirus infection.

I 322 EXTENDED LASER ABLATION OF DIFFICULT CONDYLOMAS & VULVAR INTRAEPITHELIAL NEOPLASIA (VIN). R. Reid, M.D. Greenberg, Y. Daoud, M. Stoler, Sinai Hospital of Detroit, MI & University of Rochester, Rochester, NY.

Of approximately 1,000 women referred with vulvar disease between 1983-87, the 160 most difficult cases were selected for extended laser ablation (meaning en bloc destruction of both clinical disease and adjacent acetowhitening, destroying condylomas to the papillary dermis & VIN 2-3 to the midreticular dermis). The 66 nonpregnant patients seen in 1986-87 were also treated with adjuvant topical 5 fluorouracil (5 FU) on a once weekly dosage schedule. Indications for extended laser destruction were: 84 with extensive disease (benign condylomas occupying > 30% of the vulvar surface), 34 with a refractory clinical course (failed office treatment for > 1 year) and 40 with dysplastic histology (VIN 2 or 3). Stable remission was achieved by a single laser ablation $\pm$ adjuvant 5 FU in 110. Control in the other 44 cases required repeated laser alone in 19, twice weekly 5 FU $\pm$ repeated laser in 21 and repeated laser plus thrice weekly interferon in 4 women. The remaining 4 women (3%) are still receiving interferon. Laser failure was positively correlated with: reason for selection (refractory & dysplastic > extensive), host immune status (immune suppressed or chronic illness > healthy) and HPV type (high risk > low risk). In contrast, outcome was independent of re-exposure to an untreated sex partner. In contrast, laser failure rates were significantly reduced by adjuvant 5 FU in the refractory and dysplastic group (78% vs 54%; $P > 0.05$) but not in the extensive group (83% vs 75%; NS).

I 323 HPV DNA IN CERVICAL SMEARS AND BIOPSIES AND THEIR RELATIONSHIP TO CIN AND TO CARCINOMA IN PATIENTS FROM BELGRADE (YUGOSLAVIA).

R.Grob B.Stanimirowic R.Rüdlinger M.Meandzija, Inst. for Immunol. & Virol. Zürich University CH Clinic for Obstetr. & Gynecol. Narodni Front Belgrade YU Dept. of Dermatology Zürich Univ. CH Dept of Gynecology Bern Univ. CH

94 Patients from the area of Belgrade in the age range from 20 to 62 years were studied. Cervical smears were studied. Cervical smears and/or biopsies were taken for cytological or histological analysis. HPV typing was done by Southern blotting. Cloned HPV 6/11, 16 and 18 were used as probes in hybridization experiments at low and high stringency. The studygroup encompassed 71 patients. 8 of them had CIN I, 6 had CIN II, 30 CIN III and 24 had an invasive Carcinoma. The control group consisted of 23 patients with clinically and cytologically normal cervices. In no case we were able to detect HPV 6/11 sequences. In the control group 2 of 23 were positive for HPV 16. In the CIN I group no HPV DNA could be shown. In the CIN II group 2 of 6 patients were HPV 16 positive, in the CIN III group 17 of 30 patients were HPV 16 positive, 3 were HPV 18 positive, one had an unknown type and 9 were negative. The with invasive Carcinoma showed the highest percentage of HPV presence (75%): 11 of 24 patients harboured HPV 16, one HPV 16 plus an unknown type, 4 HPV 18 and 2 showed the patterns of so far undefined types. The 6 remaining patients did not show any signals. Our Results suggest that HPV 16 and 18 are - as previous-ly seen in other areas - the most common types in high grade CIN and in invasive cervical Carcinoma in the group studied. Furthermore HPV 16 seems to be associated mainly with CIN III in younger patients whereas above 40 years it is increasingly linked to invasive Carcinoma. In this series HPV 18 tends to have more malignant potential than HPV 16 since it is associated with carcinoma in younger patients. But the number of samples is small, so this is not conclusive.

I 324 EFFECT OF MENSTRUAL CYCLE ON DETECTION OF HPV IN UTERINE CERVICAL CELLS.

F.Guijon[1], P.McNicol[2,3] E.Heywood, [2] M.Gray[2] and R.Brunham.[2] Depts. of Obstetrics, Gynecology and Reproductive Sciences,[1] Medical Microbiology,[2] University of Manitoba, R3E OW3 and Cadham Provincial Laboratory,[3] Winnnipeg, Manitoba, Canada. R3C 3Y1. We were interested in determining the effect menstrual cycle stage has on detection of Human Papillomavirus (HPV) by filter in situ hybridization (FISH) and on the adequacy of exfoliated cervical cell specimens. To this end, 519 tests were performed on non-pregnant women with abnormal Pap smear cytology referred consecutively to a colposcopy clinic at the Health Sciences Centre in Winnipeg, Canada. Each was questioned regarding the date of their last menstrual period and with informed consent, cervical cells were collected by the same gynecologist. Specimens were categorized into one of four time intervals on the basis of a twenty-eight day menstrual cycle. Laboratory personnel were blinded to the categories. Specimens were analysed for HPV infection by non-stringent hybridization with combined radiolabeled probes for HPV types 6/11 and 16/18 and autoradiography after non-stringent and stringent washing steps. Specimen adequacy was judged by a final stringent hybridization with a radiolabeled human Alu I repetitive DNA sequence. The autoradiographic signal was compared to that obtained under the same conditions with 10^5 HeLa cells. A contingency table was constructed and Chi-square analysis was carried out to test the null hypotheses- 1) Detection of HPV by FISH is not related to the stage of the menstrual cycle. 2) Adequacy of the exfoliated cervical cell specimen is not related to the stage of the menstrual cycle. The null hypotheses were accepted. HPV can be detected by FISH with equal efficiency and adequate specimens can be obtained throughout the menstrual cycle.

I 325 TREATMENT OF EXTERNAL FEMALE GENITAL CONDYLOMA ACUMINATA WITH A 0.5% PODOPHYLLOTOXIN CREAM,

Dan Hellberg, Tove Svarrer, Geo vonKrogh, Department of Obstetrics and Gynecology, Falu Hospital, Falun and Department of Venerology, Karolinska Hospital, Stockholm, Sweden

One hundred and twenty women weih external genital condyloma acuminata were randomly treated either with self application twice a day three days per week with odophyllotoxin cream (n=78), placebo cream (n=12) or by application once a week with Podophyllin (n=30). Exlusion criterias were cervical, vaginal or intraanal condylomata acuminata. Before entering the study the following examinations were made: Cultures for Chlamydia and Gonnhorrohea, wet smear of the vagina to exclude Candida and Trichomonasis, PAP-smear, colposcopy, proctoscopy and a gynecological examination. Maximum treatment lenght was 5 weeeks (perids of treatment). Of the placebo patients, one were cured, Podophyllin cured approximately 40% of the patients. Cure rate with self application of podophyllotoxin cream were after one treatment 50%, two treatments 75% and three treatments 90.7%. To calculate permanent cure rate a three month follow-up was made. Six per cent of the patients had new warts at the location of the the original at the follow up, thus giving a permanent cure rate for podophyllotoxin cream of 84% compared to 34% for Podophyllin. Side effects were only local. Burning, tenderness and pain occurred in /3 of podophyllotoxin and Podophyllin patients and in 1/3 of placebo patients. Side effects were mild to moderate, no hospitalisations and only one drop-out occurred because of the side effects. We conclude that self application of podophyllotoxin cream is both simple and more effective than Podophyllin applications.

I 326 HPV DNA IN CERVICAL CARCINOMA.ANALYSIS OF PRIMARY AND RECURRENT DISEASE,REGIONAL AND DISTANT METASTASES AND CORRELATION WITH THE CLINICAL COURSE,Hans Ikenberg, Dietmar Schwörer and Albrecht Pfleiderer,Department of Gynecology and Obstetrics,University of Freiburg,Freiburg,FRG.
The presence of HPV specific DNA in 168 primary carcinomas of the cervix,8 recurrencies,8 distant metastases and 39 regional lymph nodes was studied by Southern blot hybridization under nonstringent and stringent conditions with HPV 11,16,18,31,35.62.9% of 151 primary squamous carcinomas contained HPV 16, 2% HPV 18, 8% HPV related sequences,27.1% were negative at a sensitivity of 0.1 viral genomes/cell.In 47% of 17 adenocarcinomas of the cervix HPV 16 was identified,12% contained HPV 18,18% HPV related sequences and 23% were negative.4/8 recurrencies contained HPV 16,1/8 HPV 18 and 2/8 HPV related sequences. In 5/8 distant metastases HPV 16 was found.10/10 tumor invaded regional lymph nodes of HPV positive primary tumors contained HPV DNA as did 2/15 histologically tumor free nodes.In 1/6 tumor invaded lymph nodes of HPV negative primary tumors HPV DNA was detected while none of 8 tumor free nodes contained HPV. Adjacent histologically normal tissue of 43 cervical cancers contained HPV 16 DNA in low copy number in 3 cases.As all the corresponding tumors had a high copy number of HPV 16 a contamination cannot be ruled out.Also in this extended analysis no significant correlation between HPV type and copy number of the tumor and clinical stage,histologic type and grade of differentiation was noted.New data about the correlation between HPV status and clinical course after a mean follow uf of 30 month will be presented.

I 327 NUCLEOTIDE SEQUENCE OF HPV52b ISOLATED FROM CERVICAL CANCER, Shinichi Funahashi[1,2], Hajime Yajima[1,3] Tetsuo Noda[1] and Yoshiaki Ito[1], 1: Institute for Virus Research, Kyoto University, Sakyo-ku, Kyoto 606, 2: Toa Nenryo Kogyo Co. Saitama-ken, 454, 3: Department of Obstetrics and Gynecology, Tohoku University School of Medicine, Seiryo-machi, Sendai 980, Japan.
HPV52b DNA was isolated from cervical cancer specimen obtained from a patient in Osaka, Japan (Yajima et al. Cancer Research, in press). HPV52b was detected in 4 cases out of 31 specimens tested, while HPV16 was detected in 8 cases. We determined the nucleotide sequence of HPV52b DNA. Sequence analysis confirmed that HPV52b DNA was most highly homologous to HPV33 DNA among all the known types of HPV. The sequences of the E6 and E7 ORFs of HPV52b were compared with those of HPV6, 8, 11, 16 and 18. The result of the comparison is shown in Table below expressed as percent homology. Analysis of other part of the sequence is under way and the results will be reported.

	: 52b	33	16	18	6	11	1	8
E6	: 100	64	59	45	35	33	30	19
E7	: 100	65	59	40	51	53	34	31

I 328 HPV TYPING ON PARAFFIN EMBEDDED SECTIONS BY PCR.
A.K.N.Iversen(1),N.Pallisgaard(2),J.Bock(3),H.Jensen(4),B.Norrild(1). (1)DNA Tumor Virus Lab.,Inst.of Med.Microbiology,Uni.of Copenhagen.(2)Dept. of Molecular Biology and Plant Physiology,Uni.of AArhus.(3)Dept.of Obstetrics and Gynaecology,Rigshospitalet,Copenhagen.(4)Dept.of Pathology,Rigshospitalet, Copenhagen. DNA extracted from formalin fixed paraffin embedded (FFPE) HPV containing cells and cervical sections was analysed for HPV 6,11,16 and 18 by PCR (polymerase chain reaktion).The specificity and sensitivity of the reaction are shown by analysis of DNA extracted from sections of embedded SiHa,Caski and HeLa cells.The sensitivity of the PCR carried out on DNA from FFPE sections of the cells is compared with the sensitivity of PCR carried out on DNA extracted from a cell pellet of similar cells.Because of the small amounts of DNA available,and to increase the chance of correct amplification if DNA is damaged during extraction,we have also tried to use double-amplification.Two sets of primers were synthesized,one of which was within the frame of the other.The advantage of this system will be discussed in relationship to the size of the amplified sequence and to the number of cycles necessary for an optimal signal.Amplified sequences are identified by the presence of specific cutsites and identification of the DNA fragments by PAGE.The preliminary results of a HPV subtyping study on premalignant and malignant lesions in patients from Greenland,with the above method,will be shown.In situ hybridization of serial sections will also be presented.

I 329 **SEROREACTIVITY TO HPV 16 PROTEINS**, I.Jochmus-Kudielka[1], R.Braun[2], U. Koldovsky[3], A. Schneider[4], K.E. Schneweis[5], L.Gissmann[1], [1]Deutsches Krebsforschungszentrum, Heidelberg, FRG, [2]Inst. für Virologie, Heidelberg, FRG, [3]Universitäts Frauenklinik, Düsseldorf, FRG, [4]Universitäts Frauenklinik, Ulm, FRG, [5]Institut für medizinische Mikrobiologie und Immunologie, Bonn, FRG.
The open reading frames E4 and E7 of HPV 16 have been expressed in bacteria as fusions with the lambda CII protein (15 AA) or the MS2 polymerase (98 AA). These proteins were used as antigens in Western blot experiments to screen a total of 409 individual sera obtained from cervical cancer patients and from controls.
Only samples reacting with both fusion proteins (CII and MS2) were scored positive. The following results have been obtained:
1. 35.4% of the control sera tested reacted with the E4, 5.0% with the E7 and 2.2% with both proteins.
2. A significant difference was observed for anti-E7 reactivity between women suffering from cervical cancer and age matched controls.
Experiments are in progress to examine patients' sera for antibodies against other proteins of HPV 16 and of other HPV types.

I 330 DETECTION OF HUMAN ANTIBODIES DIRECTED AGAINST HPV 16 - ENCODED PROTEINS BY USING BACTERIALLY DERIVED FUSION PROTEINS.
Steven A. Jenison, Xiu-ping Yu, Janette M. Valentine, and Denise A. Galloway. Fred Hutchinson Cancer Research Center, Seattle WA 98104.

Bacterial fusion proteins encoded by the E4, E6, E7, L1, and L2 open reading frames of HPV 16 were used as antigen targets to detect human serum antibody reactivities. Sera from 19 women with cervical dysplasia and from 34 women attending a sexually transmitted diseases clinic were tested in Western blot assays. In the cervical dysplasia patients, 2/19 sera reacted with E4, 0/14 with E6, 2/14 with E7, 1/14 with L1, and 10/19 with L2 fusion proteins. Among the women selected randomly from the STD clinic, 3/34 reacted with E4, 1/34 with E6, 6/34 with E7, 0/34 with L1, and 14/34 with L2 fusion proteins. The locations of the HPV16 L2 and E7 immunoreactive epitopes were mapped by using nested sets of expression plasmid deletions. The DNA sequence which encodes the HPV16 L2 epitope maps to the region between coordinates 4803 and 4849. The HPV 16 E7 epitope coding sequence maps between coordinates 622 and 664. Synthetic peptides corresponding to these regions have been made and are being used in ELISAs. One human serum with a high titer of anti-HPV16 E7 antibody reactivity precipitated the E7 (18 kD) protein from lysates of radiolabelled CaSki cells.

I 331 IDENTIFICATION OF L2 OPEN READING FRAME GENE PRODUCTS OF BOVINE PAPILLOMAVIRUS TYPE-1 BY MONOCLONAL ANTIBODIES,Xian Wen Jin1,Lex M.Cowsert2,William P.Pilacinski 3,A.Bennett Jenson1;1.Department of Pathology,Georgetown University,School of Medicine, Washington,DC 20007,2.Laboratory of Tumor Virus Biology,National Institutes of Health, Bethesda,MD 20892,3.Molecular Genetics Inc.Minnetonka,MN 55343 Four hybridoma cell lines producing monoclonal antibodies (MoAb) to bovine papillomavirus type-1 L2 open reading frame (ORF) gene products have been established from mice immunized with a BPV-1 L2:B-galactosidase fusion protein. Hybridomas were selected and cloned (from over 700 hybridomas) on the basis of specific reactivity of supernatant fluids with BPV-1 L2 epitopes on disrupted BPV-1 particles and L2:B-galactosidase fusion proteins by ELISA and Western blot, and with acetone-fixed frozen sections of BPV-1 induced fibropapillomas by immunofluorescence. These MoAbs were not reactive with intact BPV-1 particles or BPV-1 L1:B-galactosidase fusion proteins by ELISA and Western blot. The 4 MoAbs detected viral structural proteins of molecular weight 76K,68K and possibly 55K in purified BPV-1 virus preparations by Western blot. Two of 4 MoAbs were cross-reactive with BPV-2 induced fibropapillomas. These findings suggest the following:1)The BPV-1 L2 ORF encodes the minor capsid proteins;2)the gene products of the BPV-1 L2 ORF have a molecular weight of 76K,68K and possibly 55K;3)minor capsid epitopes are internal to the BPV-1 particle, and 4)MoAbs reactive with genetically-engineered truncated BPV-1 L2 ORF gene products can distinguish between BPV-1 and BPV-2 productive infections.

I 332 ANALYSIS OF HPV TYPES IN ARGENTINA: HPV 6a IN A TONSILAR CARCINOMA, Tomas Kahn, Jorge A. Bercovich, Osvaldo Gonzalez Agullar, Carlos Ries Centeno, Saul Grinstein, Lutz Gissmann, Harald zur Hausen, Institut fur Virusforschung/ATV, Deutsches Krebsforschungzentrum, 6900 Heidelberg, FRG, Lab. de Virologia, Hosp. de Ninos, 1425 Buenos Aires, Argentina and Hospital Municipal de Oncologia, 1405 Buenos Aires, Argentina

We began a collaborative study to determine the prevalence of the different HPV types in genital, laryngeal and oropharyngeal lesions in patients from Argentina. The prevalence rates will be used to calculate the relative risk of HPV infection causing these cancers. In Argentina, an unexpectedly high incidence of oropharyngeal and laryngeal cancers is observed. The mortality data for these cancers approximately equals those for cancer of the cervix. These observations stimulated our studies to evaluate the possible role of HPV in oropharyngeal cancer in Argentina and to perform a corresponding analysis for cancer of the cervix.

One of the first cases included in the survey, was a 58 year old woman with a history of light smoking, consumation of mate tea and absence of prior malignancy or radiation therapy, suffering from a neck tumor. After resection it was diagnosed as a metastasis of an undifferentiated carcinoma. The primary tumor was located in the right tonsil and described as a relatively undifferentiated, infiltrating squamous cell carcinoma. Southern blot analysis of the tumor DNA revealed the typical HPV 6a PstI cleavage pattern. It was present in at least 20 copies per genome, without evidence for integration. The possible presence of an additional HPV type in this tumor is under investigation.

I 333 CHRONIC CAVITARY RESPIRATORY PAPILLOMATOSIS, Spencer W. Kerley, Carola Zalles Buchon, Jon Moran‡ James L. Fishback, Department of Pathology and Oncology and the Department of Thoracic and Cardiovascular Surgery*, Kansas University Medical Center, Kansas City, Kansas 66103. A 19 year old white male with multiple recurrences of respiratory papillomatosis was admitted for recurrent left lower lobe pneumonia with abscess formation. He was found to have a single large laryngeal papilloma, widespread bronchial papillomas and large cavitary lesions of the left lower lobe. A lobectomy was performed. The smooth walled squamous lined cavities contained many large papillomas which demonstrated strong positivity for HPV-11 by in-situ hybridization. Intranuclear viral particles were clearly evident in the papillomas by electron microscopy. Evaluation of the patient's humoral and cell mediated immunity was normal. Cavitation appears to have resulted from bronchial obstruction, post obstructive pneumonia and liquefaction necrosis. We speculate that squamous metaplasia allowed for the continued proliferation of papillomas within the cavities.

I 334 DISTRIBUTION OF HUMAN PAPILLOMA VIRUS SEQUENCES IN DIFFERENT GENITAL LESIONS. Kisseljov F.L.,Spitkovsky D.D.,Nesterova I., Zborovskaya I.,Tkeshelashvili V.* Dept.Viral Molecular Biology, Cancer Research Center, Moscow, *Georgia Cancer Center, Tbilisi, USSR.

Biopsies of human genital tracts tumours were analyzed for the presence of HPV sequences using Southern blot hybridization tecnique. Molecular clones of HPV 6a,11,16 and 18 were kindly supplied by G.Orth and H.zur Hausen. HPV 6a was identified in 2 of 48 samples (in both cases of uterus body cancer), HPV 11 - in 1 of 24 cases (also uterus body cancer) and HPV 18 - in 1 of 24 (cervical cancer). Most frequently HPV 16 sequences were detected. 15 of 54 analyzed samples were positive with variation from 30 to 75% for 2 different regions in USSR. In 2 cases of uterus body cancer two types of HPV were presented, in the first - types 16 and 11, in the second - types 16 and 6a. Physical state of HPV 16 in tumour specimens was analyzed by restriction mapping. The following types of viral genome persistance were observed - integrated form or both episomal and integrated forms.Besides that in 2 cases the results of restriction analysis allow us to suggest the presence of different types (or subtypes) of HPV related to HPV 16. The transcriptinal activity of viral genome including E6/E7 open reading frames will be presented.

I 335 IN FRAME FUSION OF ORFS E7 AND L1 IN A REARRANGED HPV-16 GENOME ISOLATED FROM A LIVER METASTASE OF A CERVICAL CARCINOMA, Heinrich G. Köchel, Andrea Otto, and Reiner Thomssen, Centre of Hygiene and Human Genetics of the University, Kreuzbergring 57, D-3400 Göttingen, West Germany
In metastases (liver, lung, pelvic lymph node, and adrenal gland) of a cervical carcinoma isolated by necropsy from a 25 year old woman HPV-16 DNA was detected in episomal and in integrated form showing the same restriction pattern in all cases. One of the integrated HPV-16 genomes was isolated from a liver metastase and analyzed by restriction mapping and nucleotide sequencing. It was found to be an internal part of a head-to-tail tandem repeat with the following features: The sequence started 5' at the BamHI site within ORF L1 followed by the NCR and ORFs E6 and E7 up to nucleotide 840 (numbering refers to Seedorf et al., Virology 145, p.181, 1985). At nucleotide 840 a sequence duplication occurred comprising the L1 ORF from nucleotide 6264 and the NCR sequence up to nucleotide 7860. This duplication led to an in-frame fusion of ORFs E7 and L1 at nucleotides 840 and 6264, respectively. Within the following section an internal deletion of 3042 Bp eliminated the ORFs E6, E7, E1 and part of the E2 ORF. This portion of the HPV-16 genome was substituted by a GC-rich DNA sequence of human origin as revealed by Southern-blot hybridization. At nucleotide 2999 the sequence continued with the normal HPV-16 gene order and stopped at the BamHI site within ORF L1.

I 336 TRANSFECTION OF TUMOUR CELLS WITH HPV16 FOR IMMUNOLOGICAL STUDIES.

Mark R.L. Krul[1], Henk L. Smits[2] and Jan D.A. van Embden[1]. National institute of public health and environmental protection, department of molecular microbiology, 3720 BA Bilthoven, The Netherlands[1]. Department of Medical Microbiology, University of Amsterdam, 1105 AZ Amsterdam, The Netherlands[2].

To study the role of the immune response to the various HPV gene products in HPV related tumours we developed an animal model. For this purpose we used an existing, chemically induced, squamous cell tumour cell line. These cells were transfected with several HPV16 recombinant plasmids, containing various parts of the HPV16 genome.
Northern blot analysis of three of the stably HPV16 transfected cell lines showed a high expression of HPV16 specific mRNA.
These transfected cell lines are still tumorigenic in normal BALB/C mice and some diffrences in in vivo growth and metastasis were observed, compared to the parental cell line.
The transfected cell lines will be used in vaccination experiments with potential vaccin candidates, like vaccinia and salmonella recombinants expressing HPV antigens. In this way it will be possible to analyse the influence of vaccinations on the immune respons to HPV antigen bearing tumour cells.

I 337 THE EPIDEMIOLOGY OF LARYNGEAL PAPILLOMAS IN DENMARK 1965-1984.

Henning Lindeberg and Ole Elbrønd, Department of Oto-Rhino-Laryngology, University Hospital, DK-8000 Aarhus, Denmark.

The incidence of laryngeal papillomas was investigated in Jutland and Funen, comprising 2,8 million inhabitants (approximately half of the danish population). The overall incidence for juvenile papillomas (<20 years at onset) was 3.62 new cases per million per year, and 3.94 cases per million per year for adult onset papillomas. Divided into 4 years intervals, the incidence of laryngeal papillomas rose from 1.27 per million per year in 1965-68 to 3.61 per million per year for the period 1969-72. Since 1969/72 the incidence has remained constant, even when the patient population is subdivided (i.e. juvenile/adult, solitary/multiple, females/males). The results are discussed in relation to current veiws on the transmission of HPV.

I 338 **HUMAN PAPILLOMAVIRUS TYPE 56, ANOTHER HPV TYPE ASSOCIATED WITH CERVICAL NEOPLASIA.** **Attila T. Lorincz, Allison P. Quinn, and Gary F. Temple. Department of Diagnostics Research, Life Technologies Inc., Gaithersburg MD.** Using low-stringency Southern blot analysis, a new human papillomavirus type was identified in a specimen of cervical intraepithelial neoplasia from a woman in the Washington, DC metropolitan area. The DNA of the HPV was molecularly cloned in λL47 and subsequently recloned into the plasmid vector pT713 for further analysis. The isolate was characterized by restriction endonuclease mapping, and its genomic organization was deduced by hybridization analysis using subgenomic fragments of HPV 6b. The DNA appears to be a typical HPV genome of 7.8 Kb which was in the form of a closed circular supercoil in the original specimen of neoplastic tissue. The clone represents a new HPV type called HPV 56 as evidenced by virtue of its complete lack of hybridization to all other known HPV types at high-stringency. Prevalence studies revealed that HPV 56 was present in 2 of 439 samples of normal cervical tissue, in 5 of 195 cervical intraepithelial neoplasia and in 2 of 56 cervical cancers. Thus, HPV 56 is a new low-prevalence HPV type associated with human cervical cancer.

I 339 THE IMPACT OF HPV TYPE AND CONCOMITANT OTHER STDs ON THE HEALING OR PERSISTENCE OF CERVICAL KOILOCYTOSIS - A PROSPECTIVE STUDY H.Luoto, M.Lehtinen, A.Ylä-Outinen, I.Rantala, U.Romppanen, P.Parkkonen, R.Aine, K.Lauslahti, P.Leinikki and J.Paavonen. The University of Tampere; SF-33101 Tampere, FINLAND.

We present follow-up data on four groups of 28 nulliparous women selected out of 191 women enrolled during years 84-86. Groups A and B consisted of patients with koilocytosis, that persisted (A) or regressed (B) during the follow-up. Group C consisted of healthy women and group D of PID-patients devoid of koilocytosis. The individual quadruplets were matched for age and residence and were followed for up to 2 years.

HPV 16 or 18 DNA was found by in-situ hybridization in 4 cases (14%) in group A and in 2 cases (7%) in group B. The cumulative rate of C.trachomatis infection by serology (seroconversion) or isolation was 43% in group A, 25% in group B, 14% in group C and 61% in group D. Corresponding figures for other STD micro-organisms (HSV, CMV, N.gonorrheae, T.vaginalis) did not differ significantly between the three first groups.

Our study indicates that in women with cervical koilocytosis concomitant C.trachomatis infection increases the relative risk (RR) of the development of persistent koilocytosis more than three-fold (RR = 3.3). Corresponding RRs were not found for the other STD micro-organisms or HPV 16 and 18.

I 341 DETECTION AND TYPING OF GENITAL HPVs USING PCR AMPLIFICATION WITH CONSENSUS PRIMERS M. M. Manos, D. K. Wright, A. J. Lewis, S. Wolinsky#, T. R. Broker* and Y. Ting, Department of Infectious Diseases, Cetus Corporation, Emeryville CA 94608; #Department of Medicine, Northwestern Medical School, Chicago, IL 60611; *Department of Biochemistry, University of Rochester, Rochester, NY 14642.

We have developed a sensitive, accurate method for the detection and typing of genital HPVs, using polymerase chain reaction (PCR) amplification. Based on regions of DNA sequence homology between HPVs 6, 11, 16, 18 and 33, a pair of consensus amplification primers was designed to yield a PCR product from the L1 region any of these viruses. The conservation of the primer regions suggests that other genital types will also serve as templates to yield analogous PCR products. Amplification products are detected by EtBr staining after gel electrophoresis, or by hybridization with an L1 consensus oligonucleotide probe. Hybridization with type-specific probes allows the identification of types 6, 11, 16, 18 and 33. HPVs have been detected and typed in genital swab samples and paraffin-embedded tissues. The method detects a broad spectrum of genital HPVs, including secondary or new types. Less than 100 copies of the HPV genome can be detected, and the typing results agree with those determined by established methods. The sensitivity of this PCR-based method, plus its ability to detect a broad spectrum of HPV types, should provide new information about the incidence of genital HPV infection and the role of these viruses in genital cancers.

I 342 COLPOSCOPIC FINDINGS IN HPV DNA POSITIVE ADOLESCENT FEMALES WITH NORMAL CYTOLOGY AND RISK FACTORS FOR CERVICAL NEOPLASIA(CIN). Barbara Moscicki, Joel Palefsky, and Gary Schoolnik. Department of Medical Microbiology, Stanford University, Stanford, CA. 94305. The utility of HPV DNA detection for CIN screening in women with normal cytology is not known. The purpose of this study was to examine in such a population the use of HPV DNA screening for detecting CIN and to identify risk factors for CIN development in a HPV positive (+) adolescent population. Methods: Females + for cervical HPV RNA-DNA hybridization test obtained on routine screening at a Teen Family Planning clinic were asked to return for interview, colposcopy, and STD testing. HPV types were grouped into 3 categories: 6/11, 16/18, 31/33/35. Results:N=20; mean age=17.9yrs. Of the 18 females with normal cytology, 10 had biopsy proven CIN/condyloma (C/C), 6 had vaginal or vulvar flat or acuminata HPV lesions; 4 had no evidence of HPV disease. Of those with C/C, 2 had type 6/11, 5:16/18 and 2:31/33/35 (1 untyped). Of those without C/C, 4 had 6/11, 3:16/18, and 2:31/33/35 (1 untyped). There were no differences between the C/C and non-C/C groups for age, yrs sexually active, no. lifetime sexual partners, contraceptive use, history of STD infection and smoking. Those with C/C had evidence of cervical immaturity in that the mean age of menarche in C/C subjects was 1 yr older than those without C/C (13.4 vs 12.4 yrs, $p<0.03$). Also, the mean area of the transformation zone measured on colpophotographs was 63% in those with C/C compared to 18% in those without. We conclude that the detection of HPV DNA may be a useful adjunct to cytology in detecting C/C. Teens with C/C had been sexually active less than 2.5 yrs suggesting that co-factors such as cervical maturity are important in the development of CIN in these youth.

I 343 **MAPPING OF IMMUNOREACTIVE EPITOPES ON HPV 16 PROTEINS BY SCREENING AN EXPRESSION LIBRARY** M.Müller, L.Gissmann Institut für Virusforschung, Deutsches Krebsforschungszentrum Heidelberg, FRG.
A 'shotgun' expression library of cloned HPV 16 DNA was prepared as follows: The DNA was sheared and partially digested with DNase I/Mn^{++} to fragments of approximately 150 bp and subsequently ligated into the PVU II site of the fd-tet-J6 expression vector, a derivative of the fd bacteriophage (Smith, G.P. 1985, Science 228, 1315-1317). Recombinants were plated on E.coli strain K91. Replica were made on NC-filters and tested by immunostaining with HPV 16 E7 rabbit-antiserum, prepared against the MS2-E7 fusion protein. 200 recombinants were found to react with the antiserum, 30 of which were further analysed by DNA sequencing. The following results were obtained:
1. all of the recombinants were shown to contain E7 specific sequences.
2. within the E7 protein two different reactive regions were localized. The first region was identified by 25 overlapping clones, represented in four groups. The minimal size for this specific epitope was 8 amino acids. A synthetic oligopeptide of this region showed to react in an ELISA using the rabbit-antisera.
The second immunoreactive site (17 AA) of the E7 protein was found in 5 clones.
Experiments are underway to test synthetic oligopeptides and the library with monoclonal antibodies as well as with human sera.

I 344 **The Prevalence of Genital Papillomavirus Infection In Women attending a Gynecological Outpatient Clinic.** *Pekka Nieminen, Valeria R.X.Soares, Antti Vaheri, Ervo Vesterinen, Jorma Paavonen, Department of Obstetrics and Gynecology, University Central Hospital and Department of Virology, University of Helsinki, Finland.*
This study was to determine the prevalence of cervical HPV infection among three groups of women attending a gynecological outpatient clinic and having different risks of exposure to HPV. Between January and October 1988 HPV DNA samples were obtained from random samples of 1) women who attended the emergency room of the outpatient clinic (Group 1 N=1060), 2) women who were seen for induced first trimester abortion (Group 2 N=289), and 3) women who were referred for abnormal Pap-smear findings (Group 3 N=196). A dot blot technique was used for the detection of HPV types 6, 11, 16, 18, 31, 33 or 35. (ViraPap and ViraType, Life Technologies, Inc. Gaithesburg. M.D.)
The prevalence of HPV DNA was 6.6% in Group 1, 13.1% in Group 2 and 38.8% in Group 3. Among randomly selected patients (Group 1 and 2) the prevalence of HPV genotypes 6/11 was 0.9%, HPV 16/18 2.2%, HPV 31/33/35 2.2%. More than one HPV genotype group was detected in 0.5% and untyped HPVs in 2.4%. The relative proportion of HPV types in Groups 1 and 2 was: HPV 6/11 8%, HPV 16/18 27% and HPV 31/33/35 27%, mixed types 7% and untyped 31%. In Group 3 the relative proportions were: HPV 6/11 21%, HPV 16/18 24%, HPV 31/33/35 24%, mixed types 10% and untyped 21%.
These results suggest high prevalence of HPV 16/18 and HPV 31/33/35 and low prevalence of HPV 6/11 among randomly selected gynecological outpatients. The significance of the differences in the prevalence of specific HPV genotypes between the groups is not yet known. The preliminary results from our prospective cohort study suggest differences in the biological behaviour of infection with different HPV genotypes.

I 345 SUPPRESSION OF THE GROWTH OF SHOPE RABBIT PAPILLOMAS WITH PMEG. Robert O. Olson, John W. Kreider, and Karla M. Balogh. Departments of Pathology, Microbiology and Immunology, The Milton S. Hershey Medical Center, Hershey, PA 17033 There are currently no satisfactory drugs for the treatment of papillomavirus infections. 9-(Phosphonylmethoxyethyl)guanine (PMEG) is a drug which is effective against experimental herpes simplex infections. We explored the possibility that this agent might be useful in the treatment of papillomavirus infections. For this purpose, New Zealand White rabbits were scarified and infected with Shope papillomavirus. PMEG treatment was begun on the day of virus infection. Treatments were given subcutaneously, twice each day. Four groups of 10 rabbits each were studied. Group A received PBS only. Groups B, C, and D were given PMEG at 0.01, 0.1, and 1.0 mg/kg bodyweight respectively. Papillomas were measured weekly in three dimensions and the geometric mean diameters were calculated. Significance of differences observed was examined with Students t-test. Periodic bodyweights and peripheral blood leucocyte counts were conducted to monitor toxicity. Some toxicity was observed in the high dose group. This was evident as epilation and about 12.5 % bodyweight loss, both of which resolved with treatment cessation on the tenth day. Peripheral leucocyte counts at 3 weeks indicated significant lymphocytosis in the middle and high dose groups. Measurement of papilloma sizes demonstrated no significant effects of the middle and lowest drug doses, but the highest dose completely suppressed the growth of most of the papillomas. We conclude that PMEG at the highest dose given was a highly effective treatment for Shope papillomavirus infections.

I 346 **COLPOSCOPIC CORRELATES OF HPV DNA,** *Jorma Paavonen, Claire E. Stevens, Nancy Kiviat, Cathy W. Critchlow, Pål Wölner-Hanssen, Timothy DeRouen, King K. Holmes, Departments of Obstetrics and Gynecology, Pathology, Medicine, and Biostatistics, University of Washington, Seattle, WA 98104.*
The recognition of HPV infections has become increasingly important. Many colposcopists have attempted to develop criteria for colposcopic diagnosis of HPV infection. However, most studies have focused on selected women with cytologic atypia likely to show abnormal colposcopic findings associated with HPV. We analysed associations of specific colposcopic features with cervical HPV among 456 randomly selected women attending an STD clinic. 11% had genital warts, 13% had koilocytosis on Pap smear, 6% were positive for HPV antigen, 11% were positive for HPV DNA, and 27% had some evidence of HPV. Logistic regression models were used to study the associations between colposcopic features and HPV DNA, adjusting for coinfections. An atypical transformation zone (ATZ)($p<0.01$), leukoplakia ($p<0.03$), and asperities ($p<0.04$) were independently associated with HPV DNA, or with any laboratory evidence of HPV. These associations were not type-specific except for leukoplakia which was mostly HPV 6/11 positive. Individual ATZ features associated with HPV DNA included opaque color tone and sharp lesion borders. The colposcopic score for HPV DNA-positive lesions was higher than for DNA-negative lesions ($p<0.01$), and higher for lesions associated with HPV 6/11 than 16/18/31. A large number of patients who were HPV-negative at the first study visit showed colposcopic features associated with HPV DNA. However, the cumulative rate of HPV DNA over time was high among such women when categorized by colposcopic findings. Our study showed that among randomly selected patients screened by colposcopy, cytology, and comprehensive microbiologic testing certain colposcopic features are associated with HPV independent of coinfections.

I 347 ANAL CYTOLOGIC ABNORMALITIES IN HIV-INFECTED INDIVIDUALS, Joel M. Palefsky, John Gonzales, Harry Hollander, Ruth Greenblatt, Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA 94305, and Departments of Pathology and Medicine, University of California, San Francisco. Recent epidemiologic studies have shown that the risk of anal cancer is 25 times higher among homosexual males than age-matched heterosexual controls. Homosexual patients with AIDS and ARC may be at particularly high risk because of high prevalence of anal human papillomavirus (HPV) infection and concurrent immunosuppression due to HIV infection. The events leading to the development of anal cancer have not yet been characterized, and therefore, it is not yet known if anal cytology has a role to play in its prevention. The purpose of this study was to characterize the cytologic abnormalities associated with anal HPV infection in homosexual males with AIDS and ARC. Eighty-two male patients underwent visual examination of the anal region, followed by insertion of a swab to the anorectal junction for cytology, and another for HPV DNA hybridization. Visual examination revealed condyloma in 10 (12%) of patients. Using pathologic criteria similar to those in use for cervical intra-epithelial neoplasia, analysis of anal cytology revealed abnormalities in 26 (32%) smears. Anal intra-epithelial neoplasia (AIN) was detected in 8 (9.6%) smears. Two of these were classified as AIN 2, and 6 were classified as AIN 1; four also contained koilocytes. Six patients (7%) were found to have condyloma acuminatum. A further 12 smears (15%) had changes suggestive of, but not diagnostic for condyloma (7 smears) or AIN (5 smears). One smear which was obtained from a patient with active Herpes simplex infection showed giant cells, and another patient with an anal fissure showed inflammatory cells. There was no correlation between external anal examination and the results of the smear. These studies show that cytologic changes which may represent anal cancer precursors are common in patients with AIDS and ARC, and that perianal examination does not correlate with intra-anal cytologic changes. HPV DNA hybridization of swab material will be presented as will the results of anoscopy and biopsy.

I 348 DETECTION OF HUMAN PAPILLOMAVIRUSES IN EXFOLIATED CERVICOVAGINAL CELLS BY IN SITU DNA HYBRIDIZATION ANALYSIS. Chia C. Pao, Chyong-Huey Lai, Shaw-Yun Wu, Kung-Chia Young, Department of Biochemistry and Obstetrics and Gynecology, Chang Gung Medecal College, Taipei, Taiwan, Republic of China

851 specimens of exfoliated cervicovaginal cells and 27 specimens of male urethral smears obtained from 706 individuals with various clinical findings were examined for the presence of human papillomaviruses (HPV) types 6/11, 16, 18, 31, and 33 by in situ deoxyribonucleic acid (DNA) hybridization analysis. The non-radioactive DNA in situ hybridization method used in this study showed no detectable cross-hybridization either among different types of HPV (except between types 6 and 11) or between the HPV DNA and human cellular DNA. Furthermore, this system was found to be very sensitive when compared with the Southern blotting method in detecting HPV. HPV was found in 233 of 276 (or 84.4%) and in 34 of 47 (or 72.3%) of cervicovaginal cells from patients with urogenital condyloma and cervical dysplasia, respectively. HPV was also detected in 6 of 39 (or 15.4%) of women with normal cytological findings who were also symptom-free. Young women who were at low risk but were infected with HPV showed significantly reduced ratios of helper/inducer to suppressor/cytotoxic T lymphocyte when compared to uninfected normal controls (1.28 ± 0.31 vs 2.47 ± 0.64; $p<0.001$). This in situ DNA hybridization method may have broad application in the screening of HPV in early lesions and in normal-looking tissues, and may be used to identify patients at risk of more serious or possibly malignant progression.

I 349 A PROSPECTIVE STUDY OF CERVICO-VAGINAL HUMAN PAPILLOMAVIRUS (HPV) INFECTION USING CERVICO-VAGINAL LAVAGE (CVL), Maire E. Percy, Terry J. Colgan, R. Michael Shier, Gordon M. Lickrish, Manju Suri, Matthew Goldsmith, Bernard Brooks, Claudia Prattinger, Depts. of Obs. & Gyn. and Pathology, University of Toronto, Toronto, Canada, M5S 1A8. This prospective study sought to correlate (i) cervico-vaginal HPV infection with the presence/absence of cervical intraepithelial neoplasia (CIN) and/or condyloma (K) and (ii) the presence of HPV infection following treatment of CIN/K. One hundred and forty-five patients with an abnormal PAP smear had a CVL performed to detect HPV infection. The cervix and vagina were washed with 10 ml of normal saline to retrieve exfoliated cells, the DNA extracted, digested with Pst I, and probed with HPV 16 using Southern blotting. All patients were colposcoped, a cervico-vaginal PAP smear taken, and any lesions biopsied. This initial assessment showed:

	no CIN/K	K	CIN I	CIN II	CIN III
HPV 16	3	1	1	2	8
HPV 31-like	0	1	1	1	1
Unknown HPV	11	7	7	7	14
HPV -ve	25	6	15	9	12
TOTAL	39	15	24	19	35

Three months after treatment, HPV infection has so far been detected in 8 of 15 patients. In 4 cases, the infection was not apparent prior to treatment. This study confirms that cervico-vaginal infection may exist without CIN/K and that such infection is frequently present following treatment of CIN and is detected by CVL. Supported by P.S.I.

I 350 CYTOPATHIC CHANGES IN HUMAN ORAL FOCAL EPITHELIAL HYPERPLASIA (FEH) Finn Praetorius, Oral Pathology, Royal Dental College, and Oral Surgery & Oral Medicine, Univ. Hosp. Copenhagen, Denmark and Per Praetorius Clausen, Pathology, Univ. Hosp. Odense, Denmark. Two different clinical types of FEH may be observed, sometimes simultaneously; 15 specimens, including both types were studied by means of light microscopy: morphology and presence of papillomavirus structural antigens (PVA) and transmission electron microscopy: morphology and presence of papillomavirus particles. The following typical morphological changes of the epithelium of FEH were registred and compared with the results of the immunohistochemical staining of PVA and the ultrastructural findings: 1. Hyperplastic rete ridges. 2. Bulging, clubbing and anastomosing of rete ridges. 3. Increased cellular density with small cells with small dark nuclei. 4. Mitosoid ballooning cells. 5. Koilocytes. 6. Type and degree of keratinization. 7. Viral particles (TEM). **RESULTS:** The occurrence of PVA does not seem to be directly related to the typical morphological changes. Koilocytes are uncommon in FEH and few are PVA positive. Mitosoid balooning cells are PVA-negative. The degree of keratinization is not proportional to the amount of PVA-positive cell nuclei in the surface layers. Viral particles were more easily found (TEM) in specimens which were strongly PVA-positive. The clinical type 1 shows the most conspicuous histomorphological changes, is often PVA-positive, and is probably younger and more active than type 2.

I 351 A COMPARISON OF BUFFERED FORMALIN WITH OTHER FIXATIVES FOR THE DETECTION OF HUMAN PAPILLOMAVIRUS DNA BY IN-SITU HYBRIDIZATION, Ralph M. Richart, MD, Gerard Nuovo, MD, Deparment of OB/GYN Pathology, Columbia University, New York, NY 10032. There is little information on whether the detection of HPV by in situ hybridization can be affected by the way inwhich the tissue is fixed. We compared the intensity of the in situ hybridization signal under low stringency conditions for several genital condylomata that contained HPV 6 or 11 which were randomly subdivided and fixed in various fixatives for 16 hours. In all cases, the largest proportion of cells with koilocytotic atypia that had detectable HPV DNA was in buffered formalin fixed tissue (80%), followd by tissue fixed in unbuffered formalin (70%), Hartman's (40%), and Bouin's solution (10%). After a high stringency wash, the greatest decrease in the overall hybridization signal was with tissue fixed in Bouin's solution. Fixation in Bouin's for 2 hours gave in situ hybridization results comparable to buffered formalin fixation but with poorer cytological detail. It is concluded that of the fixatives studied, buffered formalin is superior for the detection of HPV DNA by in situ hybridization analysis.

I 352 HPV INFECTIONS IN A GROUP OF HIV POSITIVE PATIENTS

R.Rüdlinger, R. Grob, P. Buchmann and J. Jost, Depts of Dermatology, Surgery, General Medicine of Zürich University Hospital and Institute of Immunology and Virology, CH-8091 Zürich, Switzerland

We have examined 29 HIV positive patients with HPV infections and determined the types of HPVs in various lesions, by Southern blot or by in situ hybridization. Cutaneous warts (extra-anogenital sites): These included skin warts from various sites and twice oral condylomata - a peculiarity that is otherwise only rarely seen in our department. So far twenty samples from ten patients were studied. The HPV types detected were the following: HPV 2, 3/10, 4, 6/11rel (oral condyloma) and 7. Anogenital HPV infections: All anogenital warts observed during this study were of the condyloma acuminatum type. 18 patients were studied. HPV 6/11 was detected in 15, the remainaing three had double infections with HPV 6/11 , 16, and18-related respectively. One patient suffered from HPV 16+ bowenoid papulosis (BP) with a concurrent HPV 16+ anal carcinoma. Most of our patients were in stages II and III of HIV infection and had no other cutaneous signs of impaired immunity. Hence HPV infections may be indicator lesions and should make physcians aware of the possibility of HIV infection in patients with high risk sexual behaviour. Warts in these patients should be treated as long as the lesions are limited, because it was found that condylomata acuminata especially, often were widespread and had resisted several treatments. As HIV infection is on the increase, anogenital HPV infections in these patients will continue to be a matter of considerable medical importance.

I 353 **GENITAL WARTS, OTHER STDS AND THE RISK OF VULVAR CANCER, KJ Sherman*, JR Daling*, J Chu*, AM Beckmann+, JK McDougall+. Fred Hutchinson Cancer Research Center and Depts of *Epidemiology and +Pathology, University of Washington, Seattle, WA 98195.** We have been conducting a population based study of vulvar cancer in western Washington that was designed to examine the role of sexually transmitted viruses in the etiology of vulvar cancer. To date, 166 women with in situ squamous cell vulvar cancer, 47 women with invasive squamous cell vulvar cancer, and 332 women selected as controls from the general population have been interviewed and have had serum samples collected. Women with either in situ (42%) or invasive (28%) vulvar cancer were more likely to report a past history of genital warts than were controls (4%). They were also more likely to be seropositive for HSV2 and to report a past history of gonorrhea and trichomoniasis. After adjustment for the confounding effects of exposure to other sexually transmitted diseases, number of sexual partners, age, and smoking, elevated risks remained among both case groups for genital warts (OR = 12.8, 95% CI = 5.6-29.0 for in situ lesions; OR = 15.4, 95% CI = 4.0-59.5 for invasive disease), whereas seropositivity to HSV2 was elevated only among in situ cases (OR = 2.4, 95% CI = 1.4-4.2) and the risks of trichomoniasis and gonorrhea were not elevated. In addition, tissues from these women were tested for the presence of HPV using *in situ* hybridization. One third of the 130 in situ lesions tested were positive for HPV as were 16% of the 38 invasive vulvar cancers that were tested. These data are consistent with a role for HPV in the etiology of vulvar cancer.

I 354 SERUM ANTIBODIES TO A SYNTHETIC PEPTIDE FROM TYPE 16 PAPILLOMA VIRUS

Richard S. Smith[1]; Lena Dillner[2]; Sharyn Viel[1]; Joakim Dillner[2].
[1]Johnson & Johnson Biotechnology Center, La Jolla, California; [2]Department of Virology, Karlonski Inst., Stockholm, Sweden.

Human papillomavirus (HPV) type 16 is the most common HPV type implicated in neoplasia of the cervix. We have synthesized synthetic peptides from conserved E-1 and E-2 open reading frames of type 16 HPV. One peptide labelled as 245 from the E-2 region has been shown to be immunoreactive with sera from patients with CIN as well as sera from patients with cervical carcinomas. An ELISA with peptide 245 as the solid phase antigen was used to detect an IgA and IgG response to peptide 245. We have determined that a majority (75%) of confirmed patients with CIN or CA of the cervix have a serum response to this peptide. In contrast, a minority (20%) of sera from normal controls have a response to the type 16 synthetic peptide. The serological response we detected with the ELISA was confirmed by immunoblots. Human antibodies to peptide 245 were affinity purified and immunoblotted on HPV 16 carrying cell extracts. The affinity purified antibody bound the HPV positive cell extract and controls were negative.

I 355 HUMAN PAPILLOMAVIRUS (HPV) MANIFESTATIONS IN ORAL CAVITY AND THEIR ROLE AS A POTENTIAL RESERVOIR OF GENITAL HPV INFECTIONS IN PROSPECTIVELY FOLLOWED-UP WOMEN,

Stina Syrjänen, Jari Kellokoski, Merja Yliskoski, Rauno Mäntyjärvi, Kari Syrjänen, Department of Oral Pathology; Department of Pathology; Department of Gynecology and Obstetrics; Department of Clinical Microbiology, University of Kuopio, POB 6, SF-70211 Kuopio, Finland.

To assess the role of HPV in the development of oral mucosal manifestations, as well as the role of the latter as a potential reservoir of genital HPV infections, a systematic survey of the women prospectively followed-up for HPV infections of the genital tract since 1981 (50+ 21 months) was started. So far, a series of 350 women (mean age 34+11 years) have been subjected to clinical examination of their oral status before and after 3% acetic acid application. Biopsies and scrapes from oral mucosa of all subjects were taken and analysed for the presence of HPV DNA by in situ, dot blot and Southern blot hybridization. Tiny hyperkeratotic changes were frequent in oral mucosa (73%) some of them being histologically HPV suggestive. No classical condylomas were found. Positive reaction to acetic acid application (duration over 5 minutes) was seen in 36% of the patients. However, acetowhite lesions were associated rather with changes in keratinization than with HPV infection. The scrapings were HPV DNA positive in less than 15 samples by dot blot hybridization. Southern blot analyses of the biopsies are under way. The present results suggest that oral HPV infection is rare in subjects with genital HPV infections. The results also indicate that acetowhite lesions in oral mucosa are by no means diagnostic for HPV infection.

I 356 NON-RADIOACTIVE DETECTION AND TYPING OF PCR-AMPLIFIED GENITAL HPVs,

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We have previously reported a sensitive method for the detection and typing of genital HPVs using polymerase chain reaction (PCR) amplification (Manos et al., Cancer Cells 7, in press). The method uses a set of consensus primers to amplify a ca. 450bp PCR product from the L1 region of any genital HPV type. The PCR product is detected by hybridization with a radioactive HPV consensus oligonucleotide probe, and then typed by hybridization with radioactive type-specific oligonucleotide probes. We have further developed the method to allow non-radioactive detection and typing of the PCR-amplified genital HPVs by two different approaches. 1) Using horseradish peroxidase-labeled oligonucleotides (Saiki et al., 1988, NEJM 319:537-541) for the detection and typing of PCR-amplified HPVs by a simple colorimetric reaction. 2) An immobilized probe format was developed. HPV consensus and type-specific probes were immobilized to nylon membranes through homopolymer tails (Saiki et al., in preparation). The samples to be tested are amplified with biotinylated consensus L1 primers, then hybridized to a membrane containing the battery of immobilized HPV probes. Hybridization is detected by binding HRP to the biotinylated PCR product, followed by a simple colorimetric reaction. We have used these non-radioactive methods to detect and type HPVs present in genital swabs and paraffin-embedded tissues. The sensitivity and accuracy of these methods will be compared to the results with radioactive probes.

I 357 TWO DIFFERENT HUMAN PAPILLOMAVIRUS (HPV) TYPES ASSOCIATED WITH ORAL MUCOSAL LESIONS IN AN HIV-SEROPOSITIVE MALE, Geo von Krogh[1], Stina Syrjanen[2,3], Kari Syrjanen[3] and Jari Kellkoski[2,3], [1]Department of Dermatology, Karolinska Hospital and Venhalsan at Sodersjukhuset, Stockholm, Sweden, [2]Department of Oral Pathology, Faculty of Dentistry, Unversity of Kuopio; [3]Department of Pathology, University of Kuopio, Finland

Different types of Human papillomaviruses (HPV) are associated with a variety of oral lesions. So far, HPV types 1, 2, 4, 6, 7, 13, 16, 18, 32 and 57 have been indentified in oral lesions. Immunosuppression predisposes oral mucosa to clinical manifestations of different virus infections including HPV. We describe here a 30-year old HIV-positive and immunosuppresseed man, who had suffered from oral lesions for a few months. On clinical examination, a nodular elevation was detected on the lower lip, and white keratotic areas were present on buccal mucosa bilaterally. A biopsy from the lip revealed the presence of acanthosis with a prominent granular cell layer as well as hyper- and parakeratosis. A biopsy from the buccal lesion showed a comparatively much flatter lesion with merely a basal cell hyperplasia associated with hyper- and parakeratosis. Koilocytosis was a characteristic feature in both biopsies. In Southern blot hybridization, both lesions hybridized with a probe cocktail comprising HPV 6, 11, 16 and 18 DNA under low stringency. Under high stringency, the lip lesion proved to contain HPV 7 DNA, which was also confirmed by in situ hybidization. The buccal lesion was weakly positive with HPV 11 and 13 under stringent conditions, but the restriction patterns with Pst I and Bam HI did not fit with those of any of the 57 HPV types known so far. In situ hybridizations with HPV 11 and HPV 13 probes were negative. Cloning of this "new" HPV type is currently under way.