HPV Immunology Workshop
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Sponsors:
Division of Cancer Prevention, NCI
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Workshop Summary

INTRODUCTION

Cervical cancer is the second most common cancer among women internationally. Each year, approximately 450,000 women worldwide are diagnosed with cervical cancer, and nearly 200,000 women die of the disease. Human papillomavirus (HPV) is detected in more than 99 percent of all cases of squamous cell cervical cancer, and certain HPV types are considered the primary etiologic agents of the disease. Evidence assigns significant importance to the host immune response in the pathogenesis of HPV-associated cervical lesions. This evidence, in turn, has warranted the development of HPV vaccines.

As prophylactic and therapeutic HPV vaccines are being developed, there is a recognized need to expand knowledge of the particular immunologic response associated with viral infection of the cervical mucosa. It is necessary to develop parallel research strategies for areas ancillary to HPV vaccine clinical trials to better understand mucosal immunology, better define vaccine administration routes for optimal mucosal effect, understand patterns and significance of seroprevalence, and identify surrogate markers of cellular immune response that correlate with clinical outcome. This knowledge should help scientists and clinicians optimize prophylactic vaccine programs, distinguish women who will clear HPV infections versus those who will not, and determine which women will benefit most from therapeutic vaccines and other interventions to prevent the development of cancer.

On June 29, 2002, an HPV Immunology Workshop was held, sponsored by the Department of Health and Human Services, and hosted by the National Cancer Institute's Division of Cancer Prevention. The goal of this workshop was to generate research priorities in HPV immunology necessary to further HPV vaccine development. Simply stated, the fundamental question of the workshop was: "What immunology knowledge is needed to understand the natural history of HPV infection and to define the best intervention?"

HPV IMMUNOLOGY WORKSHOP

The workshop was divided into three topic-driven sessions on mucosal immunology, seroprevalence (antibody types and subtypes), and cell-mediated markers. Each session included a series of presentations and time for general discussion. Meeting attendees also participated in one of three breakout groups that corresponded to the topical sessions.

Session I: Mucosal Immunology

Moderator and Speaker: Dr. Peggy Crowley-Nowick, Berlex Laboratories, Marion, Massachusetts

Speakers: Dr. Margaret Stanley, Cambridge University, Cambridge, England
Dr. Michael Hagensee, Louisiana State University Health Sciences Center, New Orleans, Louisiana
Dr. Philip Castle, NCI, National Institutes of Health, Bethesda, Maryland
Dr. Dianne Marais, University of Cape Town Medical School, Cape Town, South Africa

The mucosa is the host's first level of defense against HPV. It harbors all phases of HPV activity from infection to induced carcinogenesis and is distinct from the systemic environment. The goal of this session was to identify the research questions needed to better understand mucosal immunity and to better define routes of vaccine administration for optimal mucosal effect.

Exogenous Factors: Influence on Mucosal Immunity

Evidence suggests that the genital tract is a unique immune site and that cervical mucosal immunity is essential to the development and efficacy of HPV vaccines. Successful induction of peripheral antibodies may be sufficient to produce protection at the cervical level. The impact of exogenous factors, such as hormones, spermicides, and male factor contamination, must be taken into account in research endeavors.

Hormones are a major influence on mucosal immunity as they modulate cervical levels of IgA, IgG, and cytokines. Dr. Peggy Crowley-Nowick described a small study that examined immunoglobulin concentrations in cervical secretions collected daily during the follicular phase and every other day in the luteal phase. Total IgA and total IgG levels dropped dramatically at ovulation and subsequently increased. When investigators in a HPV VLP (viral-like-particle) trial examined vaccine response in relation to the phase of the menstrual cycle, anti-HPV-16-VLP IgG titer levels decreased at the time of ovulation. In oral contraceptive users, the cervical IgG response to HPV vaccine was shown to be more stable during the menstrual cycle. These findings may prove critical in vaccine development and induction of immune response.

This hormonal effect has been observed in animals. Dr. Margaret Stanley reported on the effect of hormonal status on cytokine production by lymphoid cells in the vaginas of mice at estrus and diestrus. Balb/c mice (Th2 mouse model) and C57BL/6 mice (Th1 mouse model) were sacrificed at estrus and diestrus; their vaginas were removed; and single-cell preparations were generated. In both mouse strains, the proportion of CD3+, CD4+, CD8+, and gamma-delta

TCR+ lymphocytes increased substantially at diestrus compared to estrus. The percentage of cells secreting IFN-gamma, IL-2, IL-4, and IL-10 also increased markedly at diestrus. More T cells (CD3+, CD4+, CD8+) and natural killer (NK) cells (DX5+) produced IFN-gamma at diestrus than at estrus; similarly, more CD4+, CD8+, and gamma-delta TCR+ cells produced IL-4 at diestrus than at estrus. Overall, these findings suggest that activation of T cells and NK cells is induced at diestrus and that T cell responses to pathogens may be down-regulated at estrus. Although rodents clearly have different cycles than primates, hormonal control of these cycles is important in all mammals. Immune responses in the female genital tract are under hormonal regulation and responses to pathogens may vary with hormonal cycles.

Methodological Challenges for Evaluating the Cervical Mucosa

Dr. Michael Hagensee began his presentation by pointing out that a factor critical to studying the local immune response to HPV was to improve and validate specimen collection methods. An ideal specimen collection screening tool is reproducible, is atraumatic to the cervical epithelial, will not perturb the influx of serum proteins and cells, is without contamination from vaginal secretions, allows for the collection of sufficient material, minimizes dilution, and does not affect future collections. The performance of these collections, as measured by percent recovery of immune marker, appears to depend on the composition of the absorbent material.

Collection methods for cervical specimens include brushes and wicks while collection methods for vaginal and cervical secretions include cervicovaginal lavage and tampons. A study comparing efficacy of cervical wicks versus cervicovaginal lavage for total antibodies, HPV-specific antibodies, and Th1 and Th2 cytokines showed that both products were appropriate for antibody detection, however, cytokines were not reliably detectable in cervical wick samples. In addition, preliminary results indicated degradation of IgG and IgA extracted from wick and lavage samples, thus final products may not be functional. These experiments underscore the importance of testing and validating collection methods proposed for use in clinical vaccine trials. Also key to screening and collection is ensuring that the appropriate specimen is obtained for analysis (e.g., cervical secretions, transudate).

Dr. Philip Castle concurred that methodological issues have hindered measurements of the cervical microenvironment from cervical secretions. Dr. Castle emphasized that a key concept driving the current research has been how to increase the volume of sample that can be collected and how to expand the number of measurements that can be obtained from specimens, whether the specimens are cervical swabs or secretions or other samples, given the generally small volume of material collected. One multiplex technology under evaluation to expand the use of cervical specimens for immunoprofiling is recycling immunoaffinity chromatography (RIC). RIC is a separation method in which each in a series of high-performance liquid chromatography (HPLC) immunoaffinity columns targets the isolation of a specific immunologic marker. The extracted cervical specimen is run through each of the columns from which the immunosorbed ligand is eluted, potentially testing 50 to 75 immune markers from one sample loading. Using this method to evaluate a subset of paired plasma and cervical specimens, Dr. Castle observed that cytokines generally were more detectable in plasma than in serum (e.g. IL-2), whereas chemokines were more frequently detected in cervical secretions than in plasma (e.g. regulated on activation normal T cell expressed and secreted (RANTES)). Future research will require

standardizing and validating the multiplex technologies and conducting a case-control study to examine the immunoprofiles of cervical secretions form women at different stages of cervical carcinogenesis.

The Local Mucosal Immune Response Against HPV

Dr. Michael Hagensee presented a cross-sectional study of local immune response against HPV. Low-risk HPV infections were shown to be associated with an increased Th1 balance (i.e., less IL-4, more IFN-gamma). High-risk infections resulted in no significant changes in the Th1/Th2 cytokine balance. It is unclear whether high-risk HPVs are evading the local immune system. Greater changes may be observed as the women in the study are followed over time, and Dr. Hagensee recommended longitudinal studies to better characterize Th1/Th2 cytokine balance. Dr. Margaret Stanley mentioned that there may be a role of these cytokines in downregulating HPV transcription, as has been shown for the hepatitis B virus. Limited data from studies in cancer patients suggest that IFN-gamma may downregulate the production of E7.

Study of other mucosal surfaces may add to our understanding of cervical mucosal response. Dr. Dianne Marais described studies that indicate that oral antibodies to HPV-16 confirm the presence of a common mucosal response to HPV. In one study, the majority of women with cervical neoplasia (CIN II, CIN III) and cervical cancer were found to have oral IgA antibodies to HPV-16 (R^2 for trend as the disease progressed, 0.9494). In a small study comparing prevalence of HPV-16 IgA and IgG in oral fluid, cervical mucus, and serum, oral fluid appeared to be the best predictor of HPV-16 cervical lesion, but not necessarily for HPV-11 and HPV-18. Further studies may prove oral testing and oral response to be a solid marker for some early disease. However, Dr. Marais added that assays measuring immunoglobulins in mucosal samples share some general problems. For example, concentrations can vary as a result of dilution and changes during the menstrual cycle (i.e., genital tract specimens) and neurological states (i.e., saliva). Immunoglobulin levels may also be influenced by other local infections.

Dr. Margaret Stanley noted that the primary focus for researchers should be on ensuring that the appropriate measures—whether local or systemic—are being studied. For example, phase II clinical trials of intramuscularly injected HPV-16 VLP vaccines show antibodies at the cervix; however, whether and how timing of the menstrual cycle, in addition to other factors, may affect this response must be taken into consideration.

Session II: Humoral Immune Response: Seroprevalence

Moderator and Speaker: Dr. Joakim Dillner, Lund University, Malmo, Sweden
Speakers: Dr. Raphael Viscidi, Johns Hopkins School of Medicine, Baltimore, Maryland
Dr. Michael Hagensee, Louisiana State University Health Sciences Center, New Orleans, Louisiana
Dr. Robert Burk, Albert Einstein College of Medicine, New York, New York
Dr. Richard Roden, The Johns Hopkins University, Baltimore, Maryland
Dr. Joseph Carter, Fred Hutchinson Cancer Research Center, Seattle, Washington

Prophylactic HPV vaccines target the HPV virus to prevent infection by eliciting the production of neutralizing antiviral antibodies. The goals of this session were to describe currently known

antibody types and subtypes generated in response to HPV infection and prophylactic vaccination, and to generate research questions to better understand this seroprevalence response to HPV infection and vaccination.

Dr. Joakim Dillner provided background information on research developments. Information from the past 10 years increasingly points to the importance of neutralizing antibodies against HPV and conformational epitopes in humans following natural infection; however, formal data confirming that these antibodies are important in determining type-specific immunity are lacking. Several studies, including a 15-year followup study, have demonstrated the stability of the antibody response to HPV over time. Research also indicates that some transient HPV infections fail to produce any antibody response. Most investigations of the serological responses to HPV infection have been conducted on IgG; study of the IgA response has been very limited. With renewed interest in IgA, investigations should focus on filling this gap.

HPV Serology

Serum antibodies to HPV VLPs have been shown to be markers of current and past HPV infection. However, the value of VLP antibodies as markers of immune protection against reinfection is unknown. To investigate this issue, Dr. Raphael Viscidi and his colleagues tested blood samples from three large prospective natural history studies using HPV VLP-based ELISAs to determine the association between baseline seroreactivity and the incidence of same-type DNA positivity. Subjects for Dr. Viscidi's analysis included women enrolled in the Guanacaste Study, a population-based study in Costa Rica (6,700 women followed for 5 to 6 years); the Women's Interagency HIV Study (WIHS, 2093 women followed for 1.5 years); and the HIV Epidemiology Research Study (HERS, 1254 women followed for 6 to 7 years). The results of these analyses overall suggest that serum IgG to HPV capsids, elicited by natural infection, may confer partial, type-specific protection against reinfection with HPV-16.

Dr. Viscidi outlined several limitations of these studies and analyses. First, the duration of followup and frequency of HPV DNA measurements varied across studies. Second, the number of incident infections often was low, raising the possibility of type II errors. Third, the analyses did not address HPV viral load, HPV persistence, or associated cytological abnormalities as possible effect modifiers. Prospective studies should be conducted to obtain paired isolates from initial and recurrent HPV infections for genetic analysis to document reactivation and potentially to identify risk factors and markers for reactivation.

Dr. Michael Hagensee began this talk by making several points as to why serology is the best epidemiological test available. HPV serology is a unique epidemiological tool for better understanding whom is infected with HPV as well as for better defining the immune response to this pathogen, and may be useful in offering information that Pap smears and DNA data cannot provide. First, classifications of Pap smears have changed over time. Second, neither Pap smears nor cervical swabs for HPV DNA detection are routinely archived. However, serum samples have been banked and can be used to examine HPV infection rates over time as well as geographically and by race and ethnicity. The choice of HPV serology versus cervical DNA detection depends on different factors, such as the research questions to be answered and the type of study to be conducted. Serology may be preferable for cross-sectional population-based

studies; limited data also show that prediction of cancer risk also may be best estimated using serology. Additional longitudinal studies comparing these two strategies are warranted.

Dr. Hagensee noted that most population-based HPV seroepidemiology studies focus on HPV-related disease in women with squamous intraepithelial lesions or cervical cancer. Few studies have examined community prevalence rates. He described the New Orleans Vaginal Infections and Prematurity (VIP) study, which enrolled more than 13,000 women between 1984 and 1989. The overall HPV-16 seropositive rate in the cohort was approximately 28 percent. Further study from cohorts in Oklahoma and New Orleans indicate that seroprevalence of HPV-6 and -11 have increased over time; prevalence of HPV-16 has decreased; and HPV 31 has remained relatively constant. These data may have implications for vaccine development, particularly if the rates and proportions of HPV types are representative among women across the nation.

HPV seroepidemiology studies show lower HPV seropositivity rates in men when compared with women of similar risk. One screening study of 687 high-risk men (85 percent of cohort) and women from a Sexually Transmitted Disease (STD) clinic tested for antibodies against HPV-6/11 and HPV-16 using ELISA. Results showed that women were more highly seropositive for both HPV-6/11 and HPV-16 (p level of 0.001). The reasons for this gender difference are not clear but several possibilities exist: (1) the current serology assays may not be sensitive enough; (2) separate cutpoints may be necessary for testing of men and women; and (3) with less mucosal surface to test, antigen presentation also is reduced. In the future, more HPV serology studies should include men. Dr. Hagensee noted that data from a cross-sectional study of HIV-positive men are being analyzed and that HPV status in men is being assessed in the Denver-based Project RESPECT.

Another area of investigation focuses on the lack of full concordance between HPV antibody detection in serum and cervical HPV DNA detection. Differences in results may be due to the relative insensitivity of serological assays (i.e., only 50 percent of individuals with antibodies have the same type of DNA), and to the relative insensitivity of cervical DNA detection owing to sampling errors, timing of specimen collection, and concurrent infections. Even the most highly sensitive HPV assays are only approximately 60–70 percent sensitive, despite having a high specificity. Optimization of sample collection times warrants further longitudinal study. Other sites of infection also must be taken into account as possible confounders.

HPV serology also may prove informative in defining the function of detected antibodies. Animal HPV vaccine studies suggest that serum antibodies can serve as markers of protection and that serum and mucosal antibodies can be neutralizing against the virus. Studies of naturally occurring HPV infections are investigating whether serum antibodies are reliable surrogate markers of infection and disease; whether the presence of these antibodies can prevent repeat infections; and whether a distinction can be made between functional and nonfunctional antibodies.

Polymer-Based ELISA for Detection of Anti-HPV Antibodies

However, researchers encounter several problems and limitations with the available serologic assays for HPV in their investigations. Relatively low HPV VLP antibody titers underscored the

importance of modifying and optimizing the ELISA so that background noise would be reduced; high levels of interassay and intra-assay reproducibility would be achieved; and sensitivity and specificity would be improved.

Dr. Robert Burk reported that optimization and standardization of the assay was achieved by using a combination of two novel polymer blocking agents, polyvinyl alcohol (PVA-50) and polyvinyl pyrrolidone (PVP-360), which, respectively, eliminate crossreactivity with plastic (reducing background noise) and greatly increase sensitivity. Optimization was also improved by use of only highly purified VLPs. Standardization of assays and controls across laboratories is essential, and this field of research could further benefit from development of a reproducible, validated *in situ* assay.

The Minor Capsid Protein and its Immunogenicity

Dr. Richard Roden discussed the HPV minor capsid protein and its immunogenic role. The majority of the HPV capsid is composed of 360 copies of the L1 protein located in 72 pentameric capsomeres. The L2, or the "minor" HPV capsid protein, appears to be located in the center of the pentameric capsomers at the fivefold axis of symmetry; in contrast with L1, each virion contains only 12 copies of L2. Despite its assignment as the minor capsid protein, L2 plays a significant role in facilitating viral infection and has shown promise as a prophylactic vaccinogen in multiple systems and in numerous animal studies.

L2 has many roles during infection. L2 dramatically enhances the efficiency of pseudovirion infectivity (as demonstrated *in vitro*), but it is not required for viral binding to the host cell surface. L2 antisera can completely neutralize viral infection but still permits virion binding to cell surfaces. L2 protein binds to and enters cells in the absence of L1, suggesting the presence of a surface receptor or coreceptor for this protein. The positive N-terminus of L2 can bind to DNA in a nonsequence-specific manner. Data also show that each L2 terminus is required for viral infection but not for nuclear trafficking. In addition, although most of L2 is not well conserved across HPV genotypes, portions of the protein, particularly toward the N terminus, are highly conserved; these regions may hold the greatest promise for L2-based vaccine development.

Prophylactic vaccination with L2 has been performed in a variety of animal models. Demonstrated advantages of L2 prophylactic vaccines are that L2 vaccination can induce crossneutralizing antibodies; L2 vaccination protects animals from experimental infection, even with low titers of neutralizing antibodies; and patients have tolerated a fused L2/E7 vaccination product well. Questions yet to be answered include whether neutralizing antibodies mediate protection against experimental challenge or natural exposure; determining how to overcome the poor immunogenicity of current L2 formulations; identifying crossneutralizing L2 epitopes; and determining whether L2-specific neutralizing antibodies correlate with protection in natural history studies. In regards to vaccination using a combination of L1 and L2 VLPs, it was noted that because of the strong immunodominance of L1, most of the responses to the vaccine would likely be to L1. Such experiments have not yet been conducted, however.

Unresolved Issues HPV Serology

Dr. Joseph Carter's presentation focused on three unresolved issues in the field of HPV serology: Why do some HPV DNA-positive individuals lack detectable serum antibodies? How significant is crosstype protection? And do antibodies protect from reinfection with the same HPV type?

Examination of available data suggests several reasons why a proportion of HPV-16 positive women appear to be seronegative. In some women, it takes time—often months after first detection of HPV DNA—before antibodies appear; thus, for a subset of infected women, there is a lag time before seroconversion occurs. In other cases, women may be DNA positive at only one time point, suggesting either false PCR positive or false seronegative test results. Other factors, such as viral load and genetic predisposition of the host, also may influence seroconversion.

Several studies have examined the significance of crosstype protection, with mixed results. One case-control study, for example, found that HPV-16 seropositivity appeared to have a protective effect against HPV-18-associated adenocarcinoma of the cervix. Other similar studies, including work by Dr. Carter and his colleagues, have failed to show a protective effect of HPV-6 seropositivity in, for example, HPV-16-associated cervical squamous cell cancers, in contrast with the findings of others. Future studies should examine the role of antibodies to other HPV types in relationship to cancer development and determine whether HPV protection is both type-specific and site-specific.

Very little data exist on the role of HPV antibodies in protecting against reinfection with the same HPV type. One natural history study that included an analysis of HPV-16 variants suggested that women may be protected from reinfection for several years, based primarily on evidence that women testing positive for HPV-16 did not appear to acquire new HPV-16 variants over the course of the study. Protection appeared to occur in women who generated a serum HPV-16 response as well as in women who failed to have a detectable response. In other studies, detection of HPV DNA for types 6, 16, and 31 appeared to afford protection against reinfection; however, whether the antibodies were from the original or a subsequent infection was not clear. Long-term studies are needed to determine how long protection persists and whether antibodies play a role in protection against HPV over time. If future studies do show that individuals are protected from reinfection by the same HPV type, then further investigations should focus on identifying immunologic markers or correlates of protection.

In regards to seroconversion and transient infections, Dr. Carter stated that several individuals in one study had detectable DNA for years that never seroconverted but did develop high-grade cervical lesions. The sample size of this group is so small, however, that it is difficult to draw any conclusions from these data. Followup of this cohort, including reinfection of DNA-positive nonseroconverters, continues.

Session III: Cellular Immune Response: Cell-Mediated Markers
Moderator: Dr. Margaret Stanley, Cambridge University, Cambridge, England
Speakers: Dr. Leszek Borysiewicz, Imperial College of Science, Technology and Medicine, London, England

Dr. Cosette Wheeler, University of New Mexico Health Sciences Center, Albuquerque, New Mexico
Dr. Ligia Pinto, NCI-Frederick, National Institutes of Health, Frederick, Maryland
Dr. Ian Frazer, Princess Alexandra Hospital, Woolloongabba, Australia

Therapeutic vaccines target the virally infected cell to induce cellular immune responses capable of recognizing and eliminating virally infected cells. The challenge facing researchers has been that cell-mediated markers useful for tracking other viral disease processes and for documenting vaccine effect have not correlated with resolution of HPV-induced disease of the uterine cervix. The goal of this session was to identify cell-mediated markers that can indicate clinical outcome. Markers may include, but are not limited to, markers of HPV exposure that relate to risk, markers of immunologic response to vaccine, and markers of host protection that either exist prior to HPV exposure and are innate to the host or that develop after HPV exposure.

CD8 Cytotoxic T Cell Response to Vaccinia Vaccine and Natural HPV Infection

In opening his presentation, Dr. Leszek Borysiewicz pointed out that the research in his laboratory is targeted at treatment, not prevention. Thus, the goal of the research is to destroy infected cells that are expressing a foreign viral protein. In a study on a vaccinia encoding E6 and E7 of HPV-16 and -18, HPV-specific CTLs were shown to be induced by vaccination; detected in the blood of CIN III patients but not in healthy persons; and, similarly, found in the lymph nodes and tumors of cervical cancer patients but not in healthy donors.

Evidence suggests that these T cell responses might be generated not from the tumor, but rather, through the induction of true APCs by the antigens of interest. The APCs, in turn, then must recirculate back to the tumor region. If this is determined to be the true mechanism of action, then the medical research community will need to reassess the view the CIN III as an isolated lesion. It is a dynamic lesion that appears to induce the same type of immune response in addition to reaching the lymph system as invasive carcinoma. However, Dr. Borysiewicz cautions that immunogens and assays chosen for vaccine studies must be selected very carefully.

Cellular Immune Responses to HPV VLP

Focus in Dr. Cosette Wheeler's laboratory had been on gaining an understanding of the spectrum of humoral and cellular immunity generated during natural HPV infection as well as after administration of HPV VLP vaccines. The potency of an HPV-11 VLP vaccine was evaluated at various time points in women receiving three intramuscular immunizations at months 0, 2, and 6 (n = 21 vaccine recipients, 9 controls/placebo recipients). A fourth immunization was administered to approximately one-half of the women (n = 12) at month 12. All vaccine recipients had HPV-11-specific antibody titers at month 1 and positive HPV-11 VLP-specific lymphoproliferative (LPA) responses at month 3 (i.e., 1 month after the second immunization). The magnitude of the antibody titer and the LPA were not correlated. Placebo recipients showed no LPA responses or antibody titers at any time point, as expected. The antibody and cellular immune responses were both Th1 and Th2; PMBCs from vaccine recipients secreted IL-2, IL-5, and IFN-gamma. Another ongoing phase II HPV VLP vaccine trial is examining the efficacy of vaccination over a 2-year period. Preliminary observations reveal that HPV antibody levels

return to close to baseline values within 2–3 years following final injection. Thus, a booster may be necessary to confer continued protection. Ongoing and future studies will monitor the long-term efficacy of HPV vaccines.

Dr. Ligia Pinto reported on cellular immune responses to HPV-16 L1 in healthy volunteers immunized with HPV-16 virus-like particles in a double-blind, randomized, placebo-controlled phase II clinical trial of HPV-16 L1 VLP vaccine (50 µg VLP) administered intramuscularly (without adjuvant) at 0, 1, and 6 months. The study included college-aged women reporting fewer than four sexual partners; 180 women received the vaccinations and 40 women received placebos. Cell-mediated immune responses (i.e., lymphoproliferation and cytokine production) to HPV-16 L1 VLP were evaluated in PBMCs from 43 women receiving the vaccine and 10 women receiving placebo. Vaccination increased T cell proliferative responses to HPV-16 L1 VLP at months 2 and 7 indicated by significant increases in cytokine responses (IFN-gamma, IL-5, IL-10). The greatest increases in lymphoproliferative and cytokine responses were detected at month 2 of the study. No significant increases with either lymphoproliferative or cytokine responses were reported for women in the placebo group. In sum, the results of this study indicate that the L1 HPV-16 vaccine induces strong B cell responses as well as L1-specific T cell responses characterized by proliferation and in vitro production of Th1 and Th2 type cytokines.

A Randomized Placebo Controlled Trial of a Therapeutic Vaccine for CIN

Dr. Ian Frazer described the findings of a double-blind phase I trial designed to study the safety, tolerability, and immunogenicity of the CerVax16™ vaccine. CerVax16™ is an HVP-16-based E6/E7 fusion protein combined with a saponin-based adjuvant (ISCOMs). A total of 31 women with CIN I-III were randomly assigned to receive active treatment (n = 24) with one of three doses of antigen (20 µg, 60 µg, or 200 µg) or placebo (n = 7). Up to three intramuscular injections of vaccine (20 µg or 60 µg) or placebo were given over a 6-week period; the 200-µg vaccine dose was administered at week zero only. Approximately two-thirds of the participants were HPV-16-positive at study enrollment.

Specific antibody was detected in all subjects administered the active vaccine. Twelve of 20 subjects given active vaccine showed an IFN-gamma response. CTL responses were detected in some of the actively immunized participants and in one member of the placebo group. Responses tended to increase with multiple vaccinations. No notable changes in colposcopic appearance or in cervical histology were observed. Of 14 HPV-16-positive subjects given active vaccine, 13 had lower mean HPV copy numbers per cell after the intervention. Mean viral load fell from 50 viral copies per cell pretreatment to 12 copies per cell post-treatment. Virus was undetectable in about one-half of treated initially HPV-positive subjects immediately after vaccination. Mean viral load did not change significantly in women given placebo.

BREAKOUT GROUP SUMMARIES: RESEARCH PRIORITIES

Mucosal Immunology

The breakout group focused primarily on technical issues, including optimization and standardization of collection methods and analytical assays; as well as defining more clearly the impact of exogenous factors on mucosal immune response.

I. Optimization and standardization of collection methods:
   - The larger research community needs to reach consensus on collection devices, standards for sampling the mucosal surface, and specimen storage protocols.
   - There was general agreement that collection devices such as the Wek-Cel and Merocel are the preferred devices for the collection of cervical secretions. However, further validation as well as optimization and standardization of these methods across different types of secretions (e.g., vaginal, oral, cervical) is needed.
   - There also was consensus on developing standards for assays for mucosal cytokines. Although development of a standard curve per se for cytokines is not feasible at this point, the group recommended establishing a repository of pooled cervical, vaginal, and oral secretions that could be aliquoted and then distributed to researchers to use as validated "standards." This centralized, standardized specimen repository would be designed to reduce or eliminate the variability across laboratories and analytical kits.
   - Recommendations also need to be made with respect to the types of controls, standards, and samples that should be included in various studies and assays involving mucosal collections.

II. The impact of exogenous factors on mucosal immune response:
   - Investigators need to recognize that exogenous factors play a role in the immune response of the cervix and genital tract. They need to collect relevant information, such as the sexual activities of participants at the time of and surrounding specimen collections, and incorporate such collections into study designs. Also, they need to analyze samples for factors and agents that could alter immune response.

Seroprevalence

This breakout group identified several areas for further study.

I. Prioritize examination of the usefulness of the IgA response to HPV. However, there was agreement that sensitivity can not be improved currently in cross-sectional studies of immune response.

II. Define the role of antibody production at the mucosal level.
   - Are HPV antibodies at mucosal surfaces protective? If so, which subclasses of IgA and IgG are involved?
   - Is the induction of a mucosal antibody response a requirement for an HPV vaccine?

III. Identify serological markers of primary infection and the natural history of infection and develop methods to distinguish between initial/prior and new infections. Efforts to define cases with respect to the persistence of infection over using repeated measures (i.e., of serological markers) also would prove informative.

IV. Identify better L1/L2 epitopes and serological markers of infection.

- Particularly identify L1/L2 isotopes and serologic markers that are independent of the L1 VLP antibodies. Researchers currently cannot distinguish between vaccination and prior infection. It may be instructive to consider how these and similar issues have been resolved with respect to other infections, such as hepatitis.
- Investigators also may expand studies of L2-neutralizing antibodies to include serology. There was general agreement that a solid foundation in this area exists and that future research should build upon this foundation.
- Determine if L2 antibody mediates protection in animal models, and develop more immunogenic L2-based vaccines that can induce high titers of crossneutralizing antibodies in patients.
- Conduct seroepidemiologic studies to assess whether antibody to the appropriate neutralizing epitopes in L2 correlates with protection from natural HPV acquisition.
- Test an L2-based vaccine in a prophylactic trial against all oncogenic HPV types.

V. Validate markers for different assays in vaccination studies and in studies of the natural history of HPV infection.

- Improve the HPV VLP antibody assay so that low levels of antibody can be detected. There was consensus that variability in serology is closely tied to the quality of the VLP used. Further efforts should focus on standardizing assays, measurements, and cutoff points across laboratories. Criteria should be developed for assessing "serological grade VLPs".
- Develop standards against which to quantitatively compare levels of HPV VLP antibodies. The group agreed that this should be made a high-priority recommendation in light of current and ongoing vaccine trials. International standardization of units by which antibodies are measured is urgently needed, as already recognized by the World Health Organization. A confirmatory HPV serology assay, preferably based on neutralization, also needs to be developed and standardized.

VI. Identify correlates of protection based on the humoral immune response as well as other correlates of protection (e.g., response of antibodies to various conformational epitopes, antibody affinities, subclasses of antibodies).

VII. Extend HPV serological testing to include a larger number of HPV types. Additional HPV types may be of importance not only in a medical sense but also as possible confounders of prior or current infections. Extensive serology panels would need to be established to achieve this goal.

Cell-Mediated Markers

The group made the following general research recommendation: to continue basic research studies designed to identify and assess markers.

I. The study of cell-mediated markers in the cervical immune response includes two separate components: (1) markers for natural history of HPV infection, and (2) cell-mediated immune response in neoplastic progression. The group agreed that research is not best served through the examination of end-stage cancers because the tumors already have proven to be successful. Thus, investigators need to focus on well-designed clinical trials of immunotherapeutics, including vaccines. The group also agreed that investigators should focus these efforts initially on low-bulk disease (i.e., with respect to invasive disease) and high-grade epithelial disease. There was less agreement on which proteins to study and which assays to use. Further discussion of natural history studies indicated the need for reproducible, standardized assays.

II. With respect to cell-mediated immunity (CMI), the group raised two key questions: (1) Are investigators looking at the appropriate proteins? and (2) What are the best assays for CMI? Natural history studies in particular are focused almost exclusively on E6 and E7, which may or may not be the relevant proteins of infection. Thus, investigators need to broaden their studies beyond these two proteins. Researchers also face the challenges associated with the lack of truly standardized, reproducible assays for CMI, including variability across laboratories and the assessment of low-frequency events, which, in turn, often results in examination of systemic rather than local responses. Similar issues exist with respect to the study of markers of innate immunity.

III. Consensus was reached on the following issue: the most reliable, reproducible cell-mediated assay is the delayed-type hypersensitivity assay. Although it is a systemic marker of CMI, it indicates a strong CD4 response. It also is easy to apply, which is a further benefit in terms of natural history studies. However, uniform reagents need to be developed for wide application. The group thus made the following major recommendation: a research resource that would serve as a central supplier of uniform reagents (e.g., GMP HPV proteins, DTH reagents) needs to be established for natural history studies.

IV. Ongoing research on VLP vaccines should consider the following questions:
- Do VLP vaccines confer protection against genital HPV infection and associated disease?
- What are the correlates or mechanisms of protection against HPV infection?
- What is the role of antibody and cell-mediated immunity?
- Which epitopes correlate with protection, and how long do these responses and protections last?

V. Questions and issues for future studies:
- Identify the viral antigenic targets and immune responses that protect against HPV infection and associated disease in natural history studies. Define the relationship between these factors and viral protein expression patterns.
- Identify the most sensitive methods to determine immune correlates of protection in vaccine and natural history studies.
- Identify immunologic defects throughout infection and their relationship with disease development.

- Describe the relationship between mucosal and systemic immune responses in vaccine and natural history studies.

ADJOURNMENT

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