clude changes in the carpal bones, shortening and thickening of the metacarpals, and fusions of digits (as in SPD), the phenotype is dissimilar.

The expansion of alanine tracts observed in the SPD patients is unlikely to result in a loss of function of the HOXD13 protein and is more likely to produce a protein with an altered function. Alanine-rich regions found in several fly developmentally regulated transcription factors have a role in transcriptional repression. The alanine repeats in SPD are located in the amino-terminal region of the protein, outside the DNA binding homeodomain. Interestingly, it has been shown that the N-terminal sequence of two closely related homeoproteins, Msx-1 and Msx-2 has a significant influence on repressor activity[7]. Msx-1 and Msx-2 bind to the same sequence, but Msx-1 is the more potent repressor. The repressor activity resides in the N-terminal sequence of these proteins and it is notable that the N-terminal sequence of Msx-1 contains a greater proportion of alanines (15%) than that of Msx-2 (6%). Thus alanine-rich regions may play a critical role in modulating the activity of certain homeoproteins. An interesting feature of the alanine repeats in HOXD13 is that they are not present in Hoxd13 of fish, suggesting that perhaps the insertion of alanine repeats in the N-terminal region of Hox proteins may have played a role in the acquisition of new characteristics, in this case limbs from fins. The expansion of alanine repeats in transcription factors may have been quite common during evolution. The murine Sox-3 HMG-box DNA-binding protein has several alanine repeats that are not present in the chick homologue[8]. The role of alanine repeat expansions should be easily resolved using transgenic mice. A report by Innis *et al.*[9] in this month's *Nature Genetics* confirms the role of Hoxa-13 in mouse limb development and also provides a possible insight into the mechanism of the alanine expansions in HOXD13. The authors show that the mouse limb mutation, hypodactyly, results from a deletion in the first exon of *Hoxa-13* in a region rich in CGG and CCG trinucleotide repeat tracts, including an alanine repeat. They speculate that the deletion may have been caused by non-reciprocal recombination or misalignment during replication.

The *HOXD13* mutations responsible for SPD do not alter the pattern of the digits formed, rather they appear to alter the growth rates and sizes of proximal cartilage elements (in common with mouse *Hoxd-13* null mutations). This results in a shortening of the metacarpals and metatarsals of SPD homozygotes to carpal- and tarsal-like bones (see figure). Although little is known about the target genes of HOX proteins, a possible clue to the function of HOXD13 in limb development comes from experiments showing that overexpression of the *Hoxa13* gene in chicken limbs causes the radius and ulna of the forelimbs and tibia and fibula of the hindlimbs to shorten[10]. Moreover, this phenotype appears to result from altered adhesion properties of the cells suggesting that certain cell adhesion proteins are perhaps targets of HOXD13. Cooperative interactions between HOX proteins are probably also important. In the absence of functional *Hoxa-11* and *Hoxd-11* genes (double mutants), mice develop virtually no radius and ulna, whereas these bones are much less affected in mice with mutations in either gene alone.

Now that the first link between *HOX* gene mutations and a human disorder has been made, will there be more to follow? Based on the *HOXD13* mutations it seems likely that disorders of the human axial skeleton and indeed other limb abnormalities will involve mutations in other members of the *HOX* gene family. Stay tuned!

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Preventing cervical cancer

A recent conference reiterates the need for better screening and the development of an HPV vaccine.

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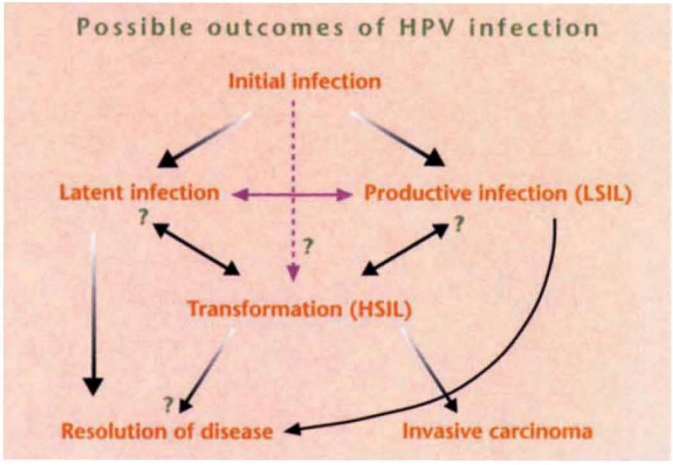
A non-Federal panel of experts recently gathered at the National Institutes of Health to develop an independent report on the current state of knowledge of the etiology, prevention and treatment of cervical cancer[1]. The consensus report was structured around several key issues: how to strengthen efforts to prevent cervical cancer, the appropriate management of low-stage and advanced stage/recurrent cervical cancer, and new directions for research efforts. This report was based on presentations by investigators working in areas relevant to the consensus questions during a two-day public session, questions and statements from conference attendees during open discussion periods that were part of the public session, and closed deliberations by the panel during the remainder of the three-day meeting.

Carcinoma of the cervix remains one of the most common cancers in women, with an anticipated 15,700 new cases and 4,900 deaths in the United States in 1996 (ref. 2). Worldwide, this malignancy continues to be a huge problem and, after breast cancer, is the second most common malignancy in both incidence and mortality, with 471,000 cases diagnosed annually. Some of the most common risk

factors for the development of cervical cancer and smoking, oral contraceptives, lower socioeconomic status, race, age, immunosuppression, sexually transmitted diseases, nutrition and early onset of intercourse.

The introduction of widespread screening programs based on the Papanicolaou (Pap) smear test in the United States and other developed countries has led to a dramatic reduction in the incidence and deaths associated with this malignancy. Because of its accessibility and well-defined typically prolonged preclinical phase, squamous cancer of the cervix is a disease where screening makes a dramatic impact. Despite the fact that Pap smears were introduced before a recognition that prospective, randomized clinical trials were the most appropriate way of documenting efficacy, there is essentially uniform agreement that the Pap smear is effective in reducing the morbidity and mortality related to cervical cancer. Even with this knowledge, substantial subgroups of American women have not been screened or are not screened at regular intervals. As a consequence of this inequality in screening patterns, one-half of the women with newly diagnosed cervical carcinoma have never had a Pap smear and another 10 percent have not had a smear in the 5 years before diagnosis. The most commonly unscreened and underscreened populations include older women, the uninsured, ethnic minorities (especially Hispanics and elderly blacks) and poor women (particularly the rural poor). One fourth of the cases of cervical cancer and 41 percent of the deaths occur in women over the age of 64 and, despite the fact that these women have the same number of physician encounters as younger women, they are much less likely to receive a Pap smear[3,4]. This situation indicates the need to educate both older women and their health-care providers about the importance of continued Pap smear screening. A critical aspect of cervical cancer prevention will be to improve outreach to underscreened populations and to identify the specific barriers to participation in screening programs for each underserved population so that appropriate interventions can be instituted.

Cervical cancer is unique in that it is the first major solid tumor that has been shown to be virally induced in essentially every case. Human papillomavirus (HPV) DNA is found in virtually all cervical cancers and precursor lesions, and multiple epidemiological studies indicate that HPV infection is the major risk factor for squamous intraepithelial lesions (SIL) and invasive cervical cancers. More than 70 types of HPV have been identified. Of these HPV types, however, only 23 have been found to infect the uterine cervix, and only one-half of these are actually associated with SIL or invasive cervical cancer. These are then further classified into low-risk types, including HPV 6 and 11, and high-risk types, most commonly 16, 18, 31 and 45, which account for more than 80 percent of all invasive cervical cancers. A recent prospective case-control study in Finland showed that women seropositive for HPV 16 antibodies carried a risk for developing cervical neoplasia 13 times that of seronegative individuals[5].



Even though it is clear that essentially all preinvasive and invasive lesions of the cervix are associated with HPV, much less is known about the other end of the spectrum, that is, the fate of women infected with HPV (see figure). There are several possible outcomes after initial HPV infection, including the development of either a latent or a productive infection. An unknown percentage of women infected with HPV will develop either low-grade SIL (LSIL) or high-grade lesions (HSIL). One-third of SIL (all grades) regress, whereas 41 percent persist and 25 percent progress. Of progressive lesions, 10 percent result in carcinoma *in situ* and one percent lead to invasive cancer. Because of the large numbers of women with HPV infection and LSIL and the potentially very high cost of treatment for these patients, it is critical that we develop reliable criteria for discriminating between lesions with a reasonable probability of progression to invasive disease and lesions that are unlikely to progress.

Despite the strong epidemiologic association between HPV infection and the development of cervical cancer, only a small percentage of patients with HPV infection will actually develop an invasive lesion. Estimates of transit times vary, but most investigators believe that it probably takes years for the progression from HPV infection to a malignant lesion. It is apparent that the virus infection alone is not sufficient for the development of cervical cancer and several possible cofactors have been proposed including: tobacco carcinogens/mutagens, coexisting viral/microbial infections, natural and contraceptive hormones, oncogene activation and dietary deficiencies. The current level of understanding is that infection with a high-risk HPV subtype is an initiating event and that additional somatic alterations associated with one or more cofactors are necessary to support malignant transformation. A recent report provides compelling evidence for the role of tobacco carcinogens as cofactors in cervical cancer. Levels of carcinogenic tobacco-specific nitrosamines were four times as high in the cervical mucus of smokers as in that of nonsmokers[6]. Women who smoke increase their risk of developing cervical cancer fourfold compared with nonsmokers.

It is estimated that as many as 5–20% of persons aged 15–49 years are infected with HPV, making this the most common sexually transmitted viral disease. The Centers for Disease Control and Prevention has estimated that nearly 1 million new cases of genital warts are diagnosed each year. Since the 1960s, the incidence of HPV infection has escalated dramatically with at least a sixfold increase in the number of physician visits each year for treatment of genital warts. The highest prevalence of HPV infection is found in sexually active women under the age of 25, with at least one study documenting as many as 40 percent of college women attending student health clinics having evidence of HPV infection. Currently, therapeutic options for this infection are expensive and of limited ef-

ficacy, usually involving destructive or excisional procedures. Significant research activity is currently focused on developing both prophylactic and therapeutic vaccines against HPV.

Therapeutic vaccine efforts are mostly based on the HPV oncoproteins E6 and E7, which are selectively retained and expressed in cervical tumors associated with HPV 16. Recently the first phase I/II clincal trial of a live recombinant vaccinia virus expressing the E6 and E7 proteins of HPV 16 and HPV 18 tested safety and immunogenicity in eight patients with late-stage cervical cancer[7]. No side effects were noted and three of eight patients mounted an HPV-specific antibody response. HPV-specific cytotoxic T lymphocytes, thought to be the most therapeutically beneficial effector mechanism, were detected in one patient. Although the study was too small to draw firm conclusions, the encouraging results warrant further investigation in a larger clinical trial.

Prophylactic vaccine development has focused on recombinant subunit preparation consisting of the L1 and L2 virion structural proteins, but recently it has been shown that the HPV L1 and L2 capsid proteins coassemble into papillomavirus-like particles when present in high levels in eukaryotic cells. A recent report by Suzich *et al.*[8] has described a successful canine model in which injection of the L1 capsid protein completely protected beagles from experimental challenge with canine oral papillomavirus. The future development of both prophylactic and therapeutic vaccines for HPV is expected to offer an effective alternative to expensive screening programs for the prevention of cervical cancer.

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Progress towards a vaccine to prevent sexual transmission of HIV

Immunization with subunit antigens prevents rectal transmission of SIV in monkeys (pages 767–775).

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Sexual transmission is the most common route for dissemination of human immunodeficiency virus (HIV) worldwide, and a vaccine capable of preventing sexual transmission might prove an effective way of halting the spread of AIDS. To be effective such a vaccine must prevent HIV transmission by three distinct mucosal routes: oral, rectal and vaginal. Each of these mucosal surfaces has a distinct mucosa-associated lymphoid tissue, and it is possible that the induction of immunity and the nature of the cellular and molecular immune responses may differ at each of these sites. Interest in developing HIV vaccines that elicit immune responses at mucosal surfaces has been spurred by studies showing that the same immunization protocols that protect monkeys from intravenous challenge with simian immunodeficiency virus (SIV) do not provide protection from vaginal SIV challenge[1]. Likewise, the SIV-vaginal infection model suggests that the route of dissemination after mucosal exposure to SIV involves infection of antigen-presenting cells (APCs, both Langerhans cells and macrophages) in the genital mucosa followed by a stepwise progression of the infection from regional lymph nodes to distant lymphoid tissues, presumably by migration of infected APCs (ref. 2, 3; see figure). These observations have led to the hypothesis that systemic immunity alone cannot prevent the genital transmission of SIV in monkeys or HIV in humans.

Two studies have shown protection of monkeys from rectal transmission after intravenous inoculation with a "subinfectious dose" of SIV (ref. 4) or intramuscular immunization with human T cell-derived whole-killed SIV (ref. 5) (although in the latter study protection may have been due to xenogeneic immune responses). In this issue of *Nature Medicine* Lehner *et al.*[6] significantly extend these earlier studies. They report prevention of rectal transmission of SIV in macaques using an SIV subunit vaccine and an immunization strategy designed to elicit mucosal immune responses. By taking advantage of the lymphatic drainage from the rectum, the authors were able to use a nonmucosal immunization route to generate strong anti-SIV mucosal immunity. A similar approach has been used in mice to elicit genital tract immunity[7], but the report by Lehner *et al.* is the first to show that immune responses generated by this route are effective against rectal transmission of SIV.

In two series of experiments macaques received a recombinant SIV vaccine (composed of envelope gp120 and core gag p27 antigens) by injection into the tissues around the iliac lymph nodes, which drain the rectal mucosa. After rectal SIV challenge, the authors report complete protection in four out of seven monkeys (and reduced virus load in the remainder) compared with infection in 13 out of 14 control animals. This is the first demonstration that protection from rectal challenge can be achieved with a vaccine composed of subunit antigens in monkeys. Earlier monkey studies used whole-killed or live-attenuated SIV vaccines but, because of safety concerns, such an approach may be unsuitable for HIV vaccines. A recent serological analysis of HIV-1 isolates from different clades shows that serum antibodies from HIV-1-infected individuals react to envelope proteins of HIV-1 isolates, which are genetically very divergent from the original infective virus[8]. This encouraging report suggests that if the right