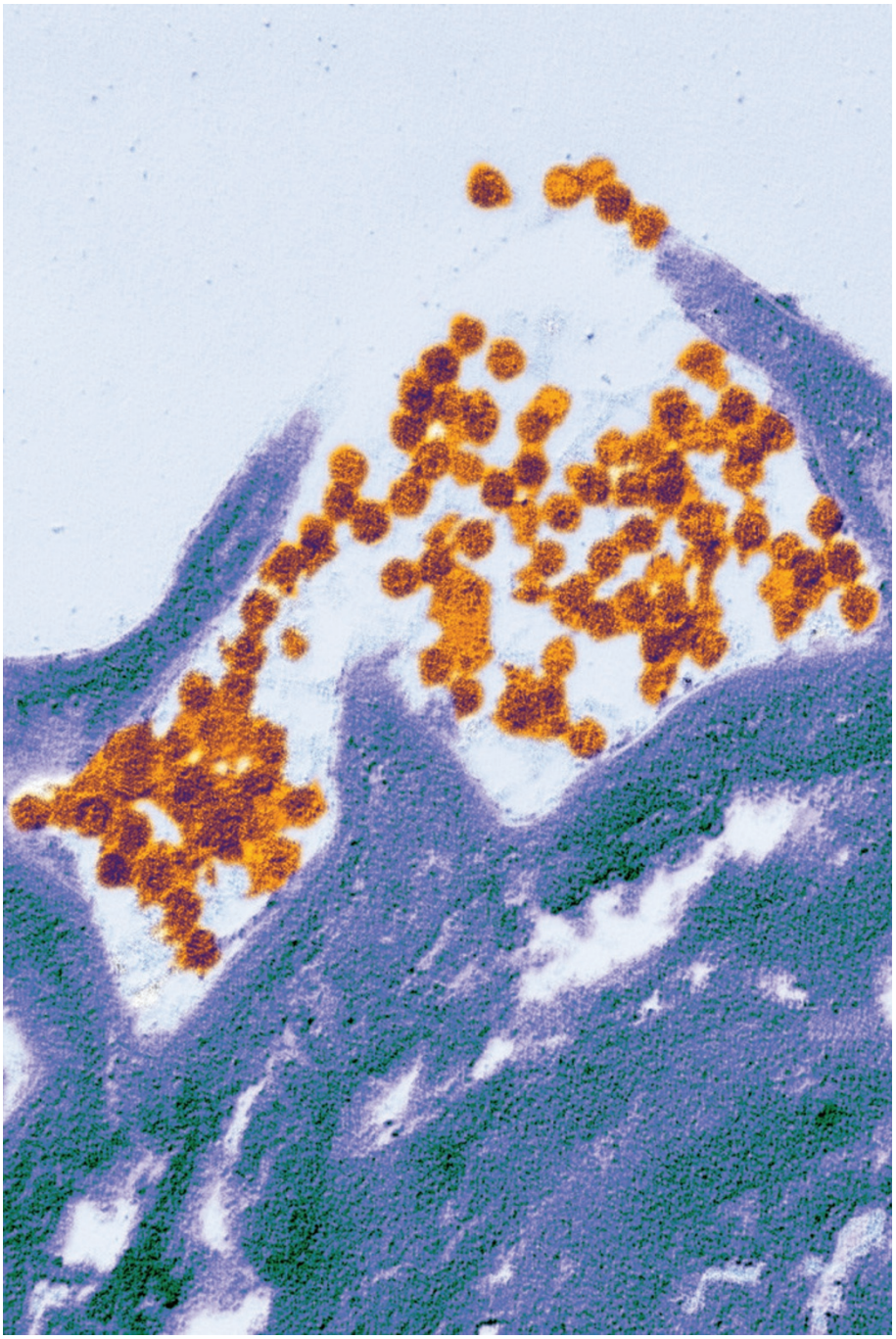

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The surface of a human wart (purple) shedding human papmillomavirus particles (brown), that can take root on another area of skin or on another person.

PATHOLOGY

Three questions

Linking specific types of HPV with cervical cancer and developing effective vaccines against should be celebrated. But there are gaps in our understanding of these viruses and how they cause disease.

BY LAURA VARGAS-PARADA

It is extremely common but has a set of peculiar features. To complete its vicious cycle of infection, human papillomavirus (HPV) has developed tricks to evade and even regulate the body's immune system. Alpha-HPVs, a genus that contains the two most dangerous HPV types, frequently infect the mucosal epithelial cells lining the surface of the cervix (see 'The global burden', page S2). Hidden inside a keratinocyte — the most common epithelial cell — HPV can remain almost invisible to the surveillance of the immune system.

This 'hitchhiker' stratagem has also hindered scientists' quest to fully understand how HPV triggers the development of cervical cancer. "The difficulties we face stem from the lifecycle of the virus," says Margaret Stanley, a virologist and epithelial biologist at the University of Cambridge, UK. This lifecycle, she explains, "is played out as the epithelium differentiates". Studying this process, she adds, "is technically very challenging because our *in-vitro* systems may not reproduce the *in-vivo* environment". The lifecycle of HPV can be viewed as a dance between the virus and the keratinocyte: in an infected keratinocyte, HPV gene expression is spatially and temporally regulated in an intricate, step-by-step performance.

Despite HPV's efforts to remain hidden, infection seems to be transient in most women. "More than 80% of all sexually active women will have seen HPV at least once, and fewer than 1% have actual problems with such an infection," says Sjoerd H. van der Burg, immunologist and head of the Experimental Cancer Immunology and Therapy Group at Leiden University Medical Center in the Netherlands. "This must mean that in most women an effective immune response is launched."

But this observation raises a major unanswered question.

1. Why do only some women develop disease?

Most women infected with HPV do not develop cervical cancer. But it remains a mystery why the immune response varies so much from individual to individual. Why do only some people develop disease? "I would rephrase the question," says van der Burg, " to what is it that does not trigger effective immunity in some patients while most others respond?" In a series of clinical studies, van der Burg's group found that women who remain healthy tend to have "well-balanced T-cell immunity" against several antigens produced by the virus. By contrast, people who develop persistent infections lack this response — their immune systems fail to recognize viral antigens[1].

T cells are part of the adaptive immune response, which springs into action once the innate immune system has done its work. But

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it is still not clear, says van der Burg, whether the response comes from $CD8^+$ T cells, which destroy HPV-infected cells, or by $CD4^+$ T cells, which help other white blood cells fight infection. This question is crucial to fully understanding how viral immunity develops.

Also unknown is how HPV escapes from the keratinocyte. Discovery of the molecular pathways that govern these processes "will probably lead to identification of genetic differences in these pathways, and hopefully to an understanding why people fail to respond appropriately", says van der Burg.

For Patti Gravitt, a molecular epidemiologist with Johns Hopkins Bloomberg School of Public Health in Baltimore, Maryland, the key question to understanding the natural history of HPV is more fundamental.

2. Does the immune response clear HPV infection?

Screening methods to determine whether someone is infected with HPV are based on detecting viral DNA in cervical swabs. "We always assumed that the presence of viral DNA in a cervical swab is equivalent to being infected; if we don't see the viral DNA then you are not infected," says Gravitt, who developed a way to detect and genotype the DNA of HPV while working at Pleasanton, California-based Roche Molecular Systems.

But this oversimplifies the situation. Although the absence of viral DNA suggests that the virus has been cleared, it could also mean that the virus has entered an undetectable persistent state known as latency. "Current assays have these methodological limitations," says Xavier Castellsagué, a cancer epidemiologist at the Catalan Institute of Oncology (ICO) in Barcelona, Spain, and director of the WHO/ICO Information Centre on HPV and Cervical Cancer. "Even if we detect some HPV DNA, we cannot tell whether it is just an inert piece of the virus or a potentially active infection," he says. "We're at the limit of HPV DNA detection." To find the answer, he explains, researchers will have to "follow up to detect persistence and early lesion development".

In most cases, a viral infection will trigger an effective immune response that clears all traces of the virus from the body. But some viruses evade the immune response. The HIV and hepatitis B viruspersist by a constant level of viral replication. Other viruses, such as the herpes simplex viruses, become latent in an apparently disease-free stage that can, under certain conditions, reactivate into a productive infection.

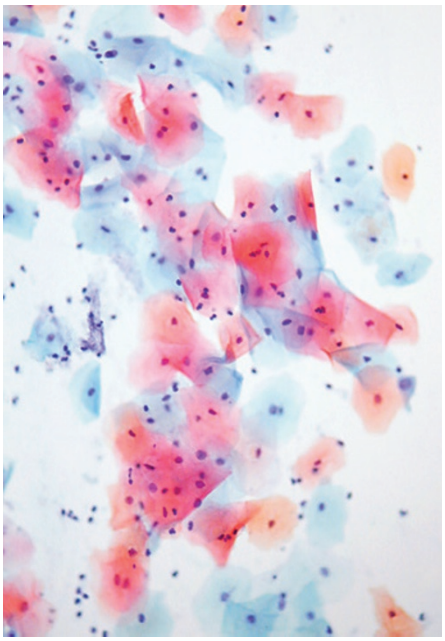
One current hypothesis is that HPV is not cleared from the body but remains in a latent state, although firm evidence for this idea is lacking. "We are working on HPV latency but are not there yet," says Ciaran B. J. Woodman, a cancer epidemiologist at the Cancer Research UK Institute for Cancer Studies at the University of Birmingham, UK.

No mechanism for latency has yet been established, and its impact on otherwise healthy women is unclear. "If you are immunocompetent, you probably have very sporadic bouts of viral replication that your immune system immediately recognizes and brings under control," says Gravitt.

"There are consequences to not knowing the answer to these questions."

Clinical observations and studies in severely immune-suppressed women, as well as in women receiving organ transplantation, seem to support this idea[2]. For now, however, technological limitations in human studies make it difficult to demonstrate HPV latency and reactivation, Gravitt says, but experimental evidence from animal models of papillomavirus infection shows that latency is occurring.

HPV could have more than one way of being latent, says John Doorbar, a molecular virologist at the National Institute for Medical Research in London. Doorbar theorizes that HPV could survive either as a persistent, asymptomatic infection or as a latent genome whose replication is controlled by the host's immune system.



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HPV-infected cells with clear cytoplasm and enlarged nuclei (purple).

In 2011, Doorbar's team studied latency in a rabbit model of papillomavirus infection. By dissecting the tissue with a laser, the researchers were able to investigate the process in unprecedented detail[3]. The group's findings "prove that papillomaviruses can persist in the site of infection after lesion clearance and that their genomes are found in the basal layer [of the mucosa] with little evidence of productive infection in the layers above", says Doorbar. Although he cautions that human papillomaviruses may not necessarily behave the same way, "for the moment we assume that they do".

But while researchers seek to pinpoint how HPV survives in the body, treating women at risk of cervical cancer raises an important question about the screening mechanism.

3. How can women at risk of HPV-related disease be identified?

Developing a good screening test requires knowledge of how long latency can last, and how differences in the nature of the infected cell can affect disease outcome. To solve those puzzles, scientists are trying to identify biomarkers for disease progression. Viral load and HPV type have both been suggested to predict the risk of developing cancer. But so far, says Woodman, robust clinical markers are lacking.

For example, certain strains of HPV are responsible for almost all the cases of malignant disease. Being infected with one of these high-risk types of HPV may be necessary for cervical cancer, but it is not enough on its own[4]. "Prognostic tests are needed to identify individuals who are at risk of progression," says Doorbar. His group is studying how gene expression patterns change in a defined way during disease progression. Such patterns, he says, will help to identify different stages of disease and help to assess the risk of progression.

A recent study, published in June 2012, identified a specific population of cells located in a particular area of the cervix where most, if not all, HPV-associated cancers arise[5]. The presence of such cells might therefore be the marker that scientists have been seeking to differentiate between benign and precancerous lesions.

On the whole, HPV has seen several successes for medical research. A virus was identified as the cause of a serious type of cancer (see 'On the case', page S16). As a result, specific screening methods were devised, and a highly effective prophylactic vaccine was developed (see 'Trial of an anticancer jab', page S4). These are rare events in public health.

But the gaps in current knowledge nevertheless provide serious barriers to further progress. "There are consequences to not knowing the answers to these questions," says Gravitt. Indeed, until the holes in the fabric of understanding are patched up, she says, it will be difficult to know "who needs to be screened, who is at risk, what a positive test result means, and who needs to be vaccinated. But the perception is that we have the problem solved." ■

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