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Abstract

Difficulties with serology for papillomavirus are associated with the large number of human papillomavirus, cross-reactions between papillomavirus, and to the diversity of lesions and target sites for infection. In addition, the expression of the papillomavirus in the superficial layers of the epithelium gives rise to the weak presentation to immunocompetent cells of viral antigens, which in turn gives rise to a weak serological response. Distinct efforts have been made in previous decades to develop more specific and sensitive serological assays. These former studies use fusion proteins and synthetic peptides, although they remain on the whole uninteresting, due to their lack of sensitivity and specificity. Only in the last few years, and principally due to the advent of various virus-like particles (VLP), have more sensitive and specific assays become available. This paper is available too at: <http://www.insp.mx/salud/index.html>

Key words: cervical cancer; VLP; HPV; serology

Resumen

Las limitaciones para la utilización de la serología para el estudio del virus del papiloma humano con fines clínicos están asociadas con la gran variedad de subtipos humanos, con las reacciones cruzadas que existen entre diversos genotipos, la diversidad de lesiones precursoras de cáncer y con los sitios blancos de infección. Asimismo, la expresión del virus del papiloma humano en las capas superficiales del epitelio dan origen a una débil presentación de células inmunocompetentes de antígenos virales, lo cual origina una elevación de la respuesta serológica. Distintos esfuerzos se han realizado en décadas previas para desarrollar ensayos serológicos más específicos y sensibles. En muchas investigaciones se ha utilizado una fusión de proteínas y péptidos sintéticos que tienen como principal limitación su escasa sensibilidad y especificidad. Sólo en los últimos años, y principalmente debido al arribo de partículas parecidas a este virus, tenemos disponibles ensayos más sensibles y específicos, ampliamente descritos en este artículo. Este artículo también está disponible en: <http://www.insp.mx/salud/index.html>

Palabras clave: cáncer cervical; VLP; virus de papiloma humano; serología

Antibodies directed against non-structural proteins

Detection of antibodies directed against the non-structural proteins E2 and E4 has been possible using fusion and synthetic proteins.¹⁻³ Antibodies against E2

protein can be detected in 2/3 viral DNA positive subjects.⁴ A study which used E2 produced in insect cells indicates that there is a correlation between the detection of anti-E2 IgA and severity of lesions, although these antibodies disappear over the course of neoplasia evolution.⁵ Unfortunately, the sensitivity and specificity

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of these assays have been evaluated in only a handful of studies. Given that there is a strong upregulation of E4 production throughout the viral cycle, anti-E4 antibody has been proposed as a marker for viral replication.^{6,7} Nevertheless, results have been inconsistent, some studies reporting several percent to 40% of positive anti-E4 subjects.⁸

It is generally accepted that E6 and E7 are produced in large quantities in cancerous cells. Consequently, anti-E6 and anti-E7 antibodies have been associated with malignant progression of the disease, and their detection is optimum in patients with cancer of the cervix.⁹⁻¹¹ These results have been confirmed using synthetic peptides, and proteins translated *in vitro* or proteins produced from a baculovirus/insect cell system.¹¹⁻¹⁷ The studies using recombinant proteins for antigen have shown that anti-E6 and E7 antibodies are detected in 50% to 60% of patients with cervical uterine cancer.¹⁸ However, not all patients are positive, and the prognostic value for detection of anti-E6 and anti-E7 antibodies is, according to the authors, either very limited or nil.^{10,15,19} The absence of immune response to these antigens does not appear to be associated with the existence of antigenic variants.¹⁷

Antibodies directed against structural proteins

In vitro culture of the virus depends on the genotype, either impossible, or too weak, and the quantity of virions present in lesions is generally too low to be used as an antigenic source. The only exception has been the preparation of HPV1 virions using plant warts, and their use for serological assays.^{20,21} Expression of structural proteins (L1 and L2) as virus-like particles (VLP₁) (Figure 1) has stimulated the development of more sensitive assays to measure immune response to the infection.²² VLP production has been achieved in several eukaryotic expression systems, including baculovirus/insect cells, vaccinia virus, Semliki forest virus, and yeast.^{22,23} These VLPs, composed of either only L1, or L1 with L2, are similar morphologically, in size, and in conformational epitopes to virions.²⁴⁻²⁶

Detection of anti-VLPs

VLPs are currently available for more than a dozen genital papillomavirus and five cutaneous papillomavirus. The most widely used technique uses VLP₁ bound to a solid support to bind serum antibody which is detected using a marked second anti-human antibody. The low sensitivity of these assays is due primarily to a weak natural immune response, rather than to poor quality antigen or assay. In order to improve specificity, one can

use a binding inhibition assay using a monoclonal antibody which recognizes the major conformational epitope of L1.²⁷ 75% to 80% of positive anti-VLP sera react with this epitope, and the majority of anti-VLP activity is directed against this epitope. However, certain subjects develop antibody to other epitopes, which means that they will not be detected with such assay. One other option to improve the specificity of assays is to neutralize anti-VLP antibody binding through pre-incubation with different VLPs followed by antibody detection.²⁸ These more laborious assays allow one to differentiate between specific and cross-reactive binding.

Humoral anti-VLP response

The anti-VLP response is type specific, despite cross reactivity with closest genotypes.²⁹⁻³³ Evidence to support this includes the fact that anti-VLP antibodies are weakly or not present in virgin women³⁴⁻³⁶ or young children.^{33,37} The different variants for genotype 16 correspond to one serotype.³⁸⁻⁴⁰

Nonetheless, there are cross reactions among phylogenetically similar papillomavirus.^{30,32,33,41-44} This has been observed between HPV 6 and 11⁴⁵⁻⁴⁸ and between HPV 18 and 45.⁴⁹ In addition, multiple cross reactions have been observed in a highly infected population, using seven genital papillomavirus.³³ Significant cross reactions have been observed between genotypes 6 and 11 (group A10), among 16, 31, 33 and

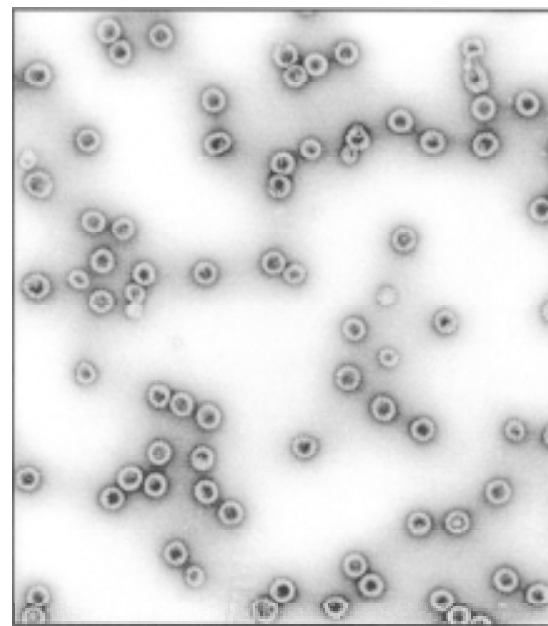


FIGURE 1. ELECTRON MICROSCOPY OF HPV 31 VLPs USED AS ANTIGEN IN ELISA TEST.

58 (group A9), and among 18, 39, 45 and 59 (group A7). It is therefore difficult to determine whether an infection is due to the test genotype or one or more of its closest genotypes.

The kinetics of anti-VLP antibody appearance is now better understood. Antibodies are found in 10 to 20% of infected individuals at the time that DNA is detectable, 1 to 3 months after infection. Prospective studies^{34,50-54} indicate that 70 to 90% of HPV 16-infected women seroconvert approximately eight months after DNA appearance. Later seroconversions may occur at 18 months, while these studies also suggest that anti-VLP antibody are always detectable in following years.⁵⁵ However, anti-VLP antibodies do not appear to persist several years after infection, and in fact seroepidemiological data indicate that they disappear with age.³⁶

Over the course of an infection, anti-VLP antibody may appear before the lesion.^{51,56,57} Anti-VLP seropositivity is also associated with lesion persistence. Approximately 80% of women who have persisting HPV-DNA, develop anti-VLP antibody although they are detected in only 22% of women who develop a transitory infection.⁵⁶

Numerous studies have been conducted regarding anti-VLP antibody detection in high risk genital papillomavirus.⁵⁸ Anti- HPV 16 capsid serum IgG is detected in 50 to 60% of women positive for type 16 papillomavirus DNA, and between 5 and 20% of control subjects.^{51,59-61}

Detection of anti-VLP antibody is also associated with the severity of lesions.⁵⁸ Anti-HPV 16 antibodies are detected in approximately 30% of patients with a low grade 'SIL', approximately 50% of severe lesions, and in 50 to 80% of cancers.⁶¹⁻⁶³ Nonetheless, anti-VLP antibody detection is less discriminatory than viral DNA detection. Prospective studies^{50,52,53} also demonstrate that anti-VLP IgG detection is more prevalent in women whose infection evolves to severe lesions, and is associated with a higher risk of developing cervical cancer.^{52,64} Therefore, during a non-persistent infection only a low percentage of subjects develop anti-VLP antibody.⁶⁵ Production of anti-VLP antibody is not a sign of healing⁵³ and in addition, detection of anti-VLP antibodies has no prognostic values for the evolution of cervical cancer.^{19,33}

Two factors influence the frequency of anti-VLP antibody detection: Sexual activity, and co-infection with HIV virus. Seroprevalence is higher in women than in men.^{66,67} This difference may be explained by the higher rate of persistent infection in women following a contaminating sexual contact. It is also recognized that papillomavirus DNA is found more frequently in subjects positive for HIV virus. However, seroprevalence in prostitutes positive and negative for HIV virus have

been reported to be the same.⁶⁸ Other studies using open population or cancer patients have found a higher prevalence of anti-VLP antibody in HIV positive subjects.^{69,70}

Significance of anti-viral capsid antibody

Serological assays for anti-VLP have no diagnostic value. Many patients who are negative for viral DNA may have been infected in previous months or years. A serological assay would be useful for this type of unapparent infection. Nonetheless, as seroconversion is slow, some subjects may be anti-VLP negative several months after the disappearance of viral DNA. In addition, during a transitory infection, only 20 to 25% of subjects develop anti-VLP antibody. Kjaer and colleagues⁷¹ found that lesions were not observed in recently infected subjects despite quickly developing anti-VLP antibodies. Although these studies were conducted on few subjects, they suggest that the appearance of anti-VLP antibody during the acute phase of the disease would be a sign of healing.

The body of evidence suggests that neutralizing antibodies are not, as in other viral diseases, a sign of healing, nor of protection against a re-infection, rather that they are the sign of a persistent replication and are particularly found in cancers associated with papillomavirus. During a natural infection, anti-viral capsid antibody titres are weak. In contrast, after anti-papillomavirus vaccination, anti-VLP antibody levels are high.

During persistent or chronic infections, an anti-VLP serological response develops in many subjects, and the prevalence of detection is positively correlated with the time course of the infection. However, antibody presence in a viral DNA carrier does not appear to be a prognostic marker. Subjects with cervical cancer and who have anti-VLP antibody have identical follow up to those without antibody, if age and size of tumors are adjusted.³³

Seroreactivity for high risk HPV is clearly a marker for sexual activity. Seroreactivity increases with total number of sexual partners.⁷² Seroprevalence in prostitutes is 10 to 14 times higher than in the general population.³⁵ Anti-VLP antibody is a more recent marker than viral DNA, which can be used in epidemiological studies to establish or confirm the role of these virus in different cancers.⁷³⁻⁷⁵ However, relative risk (OR) obtained via serology are on the order of 2 to 6, while values of 30 to several hundred are observed for DNA detection.⁷⁶ For studies on risk factors associated with infection, viral DNA detection or serology are markers with equivalent value.³⁶

Anti-VLP antibody detection is a tool to confirm the etiological role of papillomavirus. The study of

seroprevalence in different cancers is, depending on the study, to verify viral etiology for cervical, anal, vulval cancers, as well as cancer of the penis.^{41,70,73-75,77,78} The primary interest in studying anti-VLP antibodies is to measure infection prevalence in a given population, or to study risk associated with repeated infections in a same individual. The presence of anti-VLP antibody in viral DNA negative subjects is correlated with an elevated number of sexual partners.^{34,35,79} However, the presence of anti-VLP antibodies in certain young women and in children, suggests that other than sexual modes of transmission probably exist. Reactivity in these populations may be due to cross reactivity with cutaneous papillomavirus, or the existence of a genital papillomavirus infection of the oral cavity.^{80,81}

Conclusion

The body of data regarding anti-papillomavirus serology demonstrates that anti-VLP antibody detection is the only reliable serological marker. Anti-VLP antibody is largely type specific, although cross reactions occur in less than 10% of assays. Each genotype corresponds to one serotype, and it has not yet been shown whether two different genotypes can belong to a same serotype. However, the detection of anti-VLP antibodies is not a reliable assay for diagnosis, since antibody detection is strongly asynchronous with infection and is rarely the sign of healing. Serological results suggest that anti-VLP antibodies are detected primarily in association with persistent replication, and that these antibodies decrease rapidly, particularly following a non-persistent infection. In addition, these antibodies are not always present and do not persist in all infected subjects. They are therefore not a good marker of older infection. The principal application of anti-papillomavirus serology should be in years to come, that of monitoring anti-papillomavirus vaccination.

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