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Review

Measuring serum antibody to human papillomavirus following infection or vaccination

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ABSTRACT

The family of human papillomaviruses (HPVs) includes more than 130 genotypes, many of which infect the genital tract, and these can be classified as low risk or high risk for induction of genital neoplasia. Two prophylactic vaccines are currently available for the prevention of genital HPV infection: a quadrivalent (Gardasil®; Merck & Co. Inc) and a bivalent (Cervarix™; GlaxoSmithKline) vaccine. Protection against HPV infection and associated disease is observed for at least 6.4 years following immunization with the bivalent vaccine and for at least 8.5 years with the HPV 16 L1 virus-like particle of the quadrivalent vaccine. HPV vaccines induce robust immune memory, as evidenced by recall of responses after revaccination, suggesting that immunization will afford long-lasting protection. An immunological marker for ongoing protection from infection would provide information to help establish best-practice deployment of these vaccines. However, while HPV-specific antibody is likely the major mechanism of protection against HPV infection following immunization, available serological assays provide only a partial characterization of immune status, and no measured immune response has been shown to define immediate or future protection against HPV infection or associated disease. Future research efforts should therefore be directed towards correlating measures of virus-specific immune memory with continued protection against infection with the HPV types in the available vaccines, and towards determining the duration of cross-protection afforded by these vaccines against HPV types other than those incorporated in the vaccines.

Contents

Human papillomavirus

Human papillomaviruses (HPVs) constitute a large family of viruses, with over 130 genotypes. The more than 30 genotypes that infect the genital tract can be classified into either low-risk types that cause genital warts, such as HPV 6 or 11, or high-risk types that promote the development of cervical cancer, such as HPV 16 and 18 [1,2]. Characterization of the immune responses to the structural and non-structural proteins of HPVs is necessary to understand how prior infection or immunization might protect against future infection and associated disease.

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Papillomaviruses are non-enveloped viruses that have an 8-kb circular genome enclosed in a protein capsid [3]. The capsid comprises virally encoded major (L1) and minor (L2) structural proteins, both of which are synthesized late in the infectious cycle [4]. Pentamers of L1 form an icosahedral capsid shell comprised of 72 L1 pentamers, and approximately 12 molecules of L2, which are largely located inside the L1 capsid [5]. L1 sequences are highly conserved between HPV genotypes, though peptide segments, variable between genotypes, are interspersed among segments of conserved regions. The crystal structure of the virus suggests that the variable regions are primarily displayed on the outer surface of the capsid [4]. The majority of measured immune responses to HPV virions elicited by natural infection or vaccination appear to be directed to the variable regions of the L1 protein. Under appropriate conditions in vitro, HPV L1 capsid protein self-assembles into non-infectious virus-like particles (VLPs) which resemble the native virus immunologically [4], making them ideal components for HPV vaccines.

The HPV L1 VLP vaccines

Two prophylactic HPV vaccines are currently licensed: a quadrivalent (Gardasil®; Merck and Co. Inc) and a bivalent (Cervarix™; GlaxoSmithKline) vaccine. The quadrivalent vaccine is administered intramuscularly as 3 separate 0.5 mL doses; the second dose is administered 2 months after the first dose and the third dose 4 months later [3]. Each dose of vaccine consists of HPV 6, 11, 16, and 18 L1 VLPs plus the proprietary adjuvant amorphous aluminum hydroxysulfate [1]. The bivalent vaccine consists of HPV 16 and 18 L1 VLPs plus the adjuvant system AS04, comprised of aluminum hydroxide and monophosphoryl lipid A, a modified endotoxin and agonist of toll-like receptor 4 [1]. It is administered according to a 3-dose protocol at 0, 1, and 6 months.

Both vaccines provide durable protection against infection with the incorporated HPV types and associated disease, that has been observed up to 6.4 years for the bivalent vaccine [6–8] and up to 8.5 years for the HPV 16 L1 VLP of the quadrivalent vaccine [9–12]. The bivalent vaccine has demonstrated protective efficacy against cervical intraepithelial neoplasia (CIN) associated with HPV 16 and 18 when administered according to a 3-dose immunization schedule in women aged 15–25 years [6,8,13]. The quadrivalent vaccine has demonstrated protective efficacy against CIN and other anogenital preneoplastic conditions related to HPV 16 and 18 in women, and to genital warts associated with HPV 6 and 11 in women and men aged 15–26 years [9,10,14–16].

Immune response after immunization

The goal of immunization is to prevent disease following first exposure to a target pathogen in a previously uninfected subject. This is achieved by induction of a primary immune response which not only reduces the immediate infectivity of any pathogen challenge, but also enables a swift secondary immune response to the pathogen following such challenge, which can limit the capacity of the pathogen to induce disease. Following a single immunization, a primary immune response is measurable as low affinity immunoglobulin M (IgM) antibody after a period of 4–8 days [17]. This antibody response reaches a plateau between12 and 18 days after immunization and then declines. Following a second exposure to the same antigen through immunization or challenge, a further antibody response occurs, characterized by a shorter time to induction and a markedly higher level of antibody: this secondary or memory response typically also comprises antibody of a different class (immunoglobulin G [IgG]) and higher affinity for the antigen [17].

The currently available VLP-based HPV vaccines present conformational epitopes found on the natural HPV virus and prime the immune system to generate antibodies which can neutralize the virus and help prevent infection and disease following HPV challenge [18].

VLPs are highly immunogenic. HPV immunization induces peak geometric mean antibody titers that are 80- to 100-fold higher than those observed following natural infection [19]. Furthermore, after 18 months, mean vaccine-induced antibody titers remain 10- to 16-fold higher than those recorded with natural infection [19], and these levels appear to be preserved over time, suggesting that immunization may provide long-term protection against infection. Neutralizing antibody to papillomavirus is sufficient to prevent infection following challenge with virus in animal models, though persistence of measurable antibody may not be necessary to prevent disease following exposure in humans.

Anti-HPV antibody measurements

Measurable immune responses to viral proteins in humans following proven HPV infection are weak and inconsistent, and the mechanism and extent of any immune protection against reinfection with HPV following natural infection is unknown, though primary infection in cattle and dogs with their papillomaviruses prevents disease following subsequent challenge. Following immunization with HPV, measurable immune responses to the viral capsid are consistently observed. However, no immune responses have been defined that correlate with protection against infection or disease following subsequent natural exposure to HPV [1]. A surrogate marker for protection against HPV infection or disease following exposure to HPV in previously immunized or naturally infected individuals would assist in developing effective immunization protocols, define any requirement for booster immunization, and determine the utility of any cross-protection induced to HPV types not in the current vaccines [14]. Such a marker would also facilitate introduction of new HPV types into future HPV vaccines.

Definition of an appropriate HPV serological assay to monitor the response to natural infection and immunization is particularly challenging given that a portion of naturally infected and of vaccinated individuals do not become seropositive to any currently available assay, and yet are apparently protected against disease following further infection [18]. An evaluation of the correlation between quadrivalent vaccine-induced serum anti-HPV responses and efficacy in 17,622 women could discern no correlation between antibody levels and HPV infection or disease due to the low number of disease cases. After up to 48 months of follow-up, overall vaccine efficacy was 98.4%. Though 40% of vaccine recipients lacked measurable antibody to HPV 18 at 48 months, there were no cases of HPV 18 infection or related disease in vaccine recipients, but there were 26 cases in the unimmunized control group. These findings indicate that the protection conferred by immunization occurs with HPV 18 antibody titers that are below detectable limits [14]. This protection could either be due to recall by infection of a protective memory B-cell response, to failure of current antibody assays to detect the appropriate antibody type or specificity, or to a non-antibody-dependent mechanism of immune protection involving T-cell memory.

The pivotal clinical trials evaluating the two currently licensed vaccines have used different methods to evaluate virus-specific IgG antibody titers after immunization, and therefore serological data are not directly comparable between the studies. The quadrivalent vaccine trials used a competitive Luminex® assay (cLIA) or a competitive radioimmunoassay (cRIA), which uses multiplex technology to detect defined type- and epitope-specific antibodies against HPV 6, 11, 16, and 18 VLPs [18,20]. The different binding affinity of each monoclonal antibody for its epitope on the VLP, and the different proportion of the available epitopes represented by the antibodies used for different HPV types, preclude valid comparisons of magnitude of measured immune response to different HPV types for these assays. The bivalent vaccine trials used an enzyme-linked immunosorbent assay (ELISA) technique utilizing VLPs as substrate [19]. ELISA

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measures total serum anti-VLP IgG antibody to all epitopes presented by the VLPs. It does not discriminate between type-specific conformational antibodies and type-common antibodies, which tend to be specific for epitopes presented by denatured L1 and which are rarely neutralizing [20]. None of the essays examines antibody affinity or avidity, nor determines the in vivo neutralization potential of serum, though the cLIA and cRIA assays can be designed to be specific for antibodies recognizing some of the virus epitopes that bind known neutralizing antibody. None of the essays distinguishes vaccine-induced antibody to one HPV type from antibody raised against other HPV types by vaccination or natural infection [20]. Thus, without a demonstrated correlate of immune response in either assay with clinical protection, none of the essays can be said to be useful for measuring the effectiveness of vaccination. However, each assay can be used to provide data on relative immunogenicity of the same vaccine product in different patient populations, which is important for bridging studies designed to validate the use of immunization in populations (e.g. children prior to onset of sexual activity) that, because of the low incidence of disease, are not amenable to conventional clinical efficacy studies.

Type specificity of neutralizing antibodies

Immunization with VLPs can protect individuals from subsequent exposure to infection. Protection against papillomavirus infection can also occur via passive transfer of serum antibodies between vaccinated or naturally infected animals and naive animals, demonstrating that specific antibodies are sufficient to give protection. Immunization with HPV VLPs predominantly produces type-specific virus neutralizing antibodies, though some cross-protection against other related HPV types is observed, both in preclinical studies and in clinical trials [1,5,21]. In particular, cross-reactivity has been observed between HPV 16, 31, 33, and 58 and between HPV 18 and 45 [1], indicating that immunization with one HPV type may offer some degree of cross-protection against others [1]. However, cross-neutralization elicited by L1 VLPs represented <1% of the neutralizing activity induced by the dominant conformational epitopes, and the affinity and duration of such cross-neutralizing antibody is currently unknown.

Immune memory

Following natural HPV infection, serum antibody levels to HPV 16 remain stable over more than 4 years of follow-up [22]. Serum antibody levels are routinely used to monitor vaccine efficacy. However, following immunization, there are few data on the regulation and maintenance of antibody levels over long periods of time in the absence of constant antigenic stimulation [23].

Humoral immune responses to infection or immunization are characterized by an initial IgM response, and subsequent production of IgG of increasing affinity for the relevant antigen, due to selection of higher avidity B-cell clones following somatic hypermutation of antibody genes. Antibody production is largely by plasma cells located in lymph node germinal centers and in mucosal tissues at sites of inflammation. The majority of these cells have a limited lifespan, but some persist, ensuring ongoing production of low levels of antibody over the lifetime of the animal. In addition, an expanded population of antigen-specific memory B and T cells are produced following immunization that can more rapidly respond to antigen following a further exposure to the same initial pathogen [23,24]. Immune memory is the basis for long-term protection against disease following immunization: high-affinity B-cell memory develops under the guidance of helper T cells [1]. Following further antigen exposure, these antigen-specific B and T cells undergo extensive proliferation, rapidly producing high-affinity antibody to prevent spread of the relevant pathogen.

Following infection or immunization, peak serum antibody levels are reached within a month, decline over subsequent months, and are then maintained at constant levels for long periods of time [23]. Smallpox vaccine-specific memory B cells can be detected ≥60 years after immunization; significantly, antibody levels have been shown to have remained stable between 10 and 60 years after pathogen exposure [25]. However, the exact mechanism by which serum antibody levels are maintained over a long period of time in the absence of antigenic stimulation remains to be determined [23]. Preclinical evidence indicates that several distinct mechanisms may contribute to sustained antibody levels, including the longevity of a subset of antigen-specific plasma cells, continuous antigenic stimulation of memory B cells by small amounts of antigen persisting in specialized antigen-presenting cells in the lymph node [23], and slow homeostatic activation of all memory B cells without antigen exposure, leading to ongoing low level production of antibody [23]. Further research is necessary to determine whether different vaccines elicit different memory responses.

A study in 552 women aged 16–23 years indicates that the quadrivalent HPV vaccine induces robust immune memory [26]. Following a 3-dose regimen, serum anti-HPV levels declined postimmunization, reaching a plateau at 24 months. In a subset of women followed for 60 months, serum anti-HPV levels remained stable; 1 week and 1 month after administration of a challenge dose of quadrivalent vaccine, anti-HPV levels remained higher than those observed following the initial immunization series [26].

Future perspectives

The best evidence of induction of effective long-term immunity following immunization is ongoing protection against disease. Current data report promising results regarding the durability of HPV vaccine-induced protection, with ongoing efficacy demonstrated over periods of up to 8.5 years. HPV vaccines induce robust memory immune responses which should ensure long-lasting protection. Future research efforts should therefore be directed towards correlating measures of virus-specific immune memory with continued protection against infection with the HPV types in the available vaccines, and towards determining the duration of cross-protection afforded by these vaccines against HPV types other than those incorporated in the vaccines.

Key points

- HPV vaccines induce virus-specific antibody, sufficient to protect against HPV-associated disease, though antibody may not be the sole mechanism of protection.
- Antibody to conformational epitopes on the viral capsid is more effective for virus neutralization than antibody to linear or denatured capsid protein.
- Humoral immune responses to HPV measured by currently available assays do not predict individual protection against disease.
- Following vaccination, immune memory is an important component of ongoing protection.
- HPV vaccination induces some cross-protective immunity against other HPV types, but the significance for long-term protection of cross-reactive antibody is unknown.

Disclosure

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Conflict of interest statement

I.H.F. receives royalties from the sale of HPV VLP-based vaccines.

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