Immune responses to human papillomavirus

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Abstract

The immune system uses innate and adaptive immunity to recognize and combat foreign agents that invade the body, but these methods are sometimes ineffective against human papillomavirus (HPV). HPV has several mechanisms for avoiding the immune system. HPV infects, and multiplies in keratinocytes, which are distant from immune centers and have a naturally short lifespan. The naturally short life cycle of the keratinocyte circumvents the need for the virus to destroy the cell, which would trigger inflammation and immune response. In addition, HPV downregulates the expression of interferon genes. Despite viral immune evasion, the immune system effectively repels most HPV infections, and is associated with strong localized cell mediated immune responses. New prophylactic L1 virus-like protein vaccines for HPV 16 and 18 and HPV 6, 11, 16, and 18 are in phase 3 trials. Available data suggests that these vaccines are safe, produce high levels of antibodies, and are effective at preventing HPV infection.

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1. Introduction to host immunity

The world is a dangerous place in which Homo sapiens must continuously utilize sophisticated, flexible, and lethal defenses to rebuff the massed regiments of viruses, bacteria, and eukaryotic parasites. Host defense is a partnership between innate immunity (phagocytes, soluble proteins [e.g., cytokines, complement, and epithelial barriers]) and adaptive immunity (antibody, cytotoxic effector cells). The innate immune system detects the pathogen and acts as the first line of defense, clearing the majority of microbial assaults. Innate immunity has no specific memory, but is responsible for activating adaptive immunity. The adaptive immune response generates exquisitely specific lethal effector responses to foreign antigens as well as long-lived cells with memory of the insult. Antibody-mediated humoral immunity clears free virus particles from body fluids and can prevent viral reinfection, while cell-mediated immune responses are essential for the clearance of virus-infected cells and the generation of immune memory.

Innate immunity is activated by cell injury or cell death and manifests as inflammation, the local vascular response to injury. During inflammation, soluble and cellular innate immune effectors are recruited. Local parenchymal cells are recruited and local phagocytes are then activated to secrete inflammatory cytokines and other defense molecules. Crucially, dendritic cells, the only antigen-presenting cells (APCs) that can activate naïve T lymphocytes, are activated to kick-start the adaptive immune response.

Innate immunity uses proteins encoded in the germ line to identify potentially noxious elements. Adaptive immunity, in contrast, is found only in vertebrates, is almost infinitely flexible, and is based on T and B lymphocyte receptors generated by somatic gene rearrangement during ontogenesis. Neither the specificity of these receptors nor the response that might be induced in the lymphocytes after the ligation of the receptor by antigen is predetermined; these responses are determined by coordinated efforts between the innate and adaptive immune systems. The innate immune response generates the signals that identify the nature of the antigen and the appropriate type of effector response. For example, the germ line-encoded receptors of the innate immune system recognize conserved molecular targets that are essential products of microbial physiology and central to microbial survival. These
Th1 cells secrete IFN-\(\gamma\) and Th2 cells secrete IL-4 and IL-10 (and other cytokines). Two major subsets of CD4+ T cells known as Th2 or Th1 cells.

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Specific lymphocytes are difficult to activate by antigen alone. The interaction between the T cell and the APC is very complex, since naive antigen-association into the class II complex. The interaction between the T cell and the APC is very complex, since naive antigen-specific lymphocytes are difficult to activate by antigen alone. Thus, several other receptor/ligand interactions must occur in a regulated order before the T cell is activated and starts to proliferate into armed effector T cells.

CD4+ T cell activation results in the secretion of a variety of small proteins, or cytokines, that help and regulate other cells. The pattern of cytokine expression defines the two major subsets of CD4+ T cells known as Th2 or Th1 cells.

- Th2 cells secrete IL-4 and IL-10 (and other cytokines) and help antigen-primed B lymphocytes differentiate into plasma cells and secrete antibodies, the effector molecules of humoral responses.
- Th1 cells secrete IFN-\(\gamma\) and create a milieu in which key cytotoxic effectors—macrophages, natural killer cells, and cytotoxic CD8+ T lymphocytes—are activated, generating cell-mediated immunity.

A third category of T cells, regulatory T cells (Tregs) with the phenotype CD4+CD25+, expresses the signature transcription factor Foxp3 and usually secretes IL-10 and TGF-\(\beta\). Cells with this phenotype are thought to recognize self-antigens and function to prevent autoimmunity; however, they also regulate responses to exogenous antigens, and have been implicated in chronic and immunopathologic viral infections [2].

The APC expresses receptors and secretes local cytokines that dictate whether the T cell takes the Th2, Th1, or regulatory path. These functions of the APC are activated by receptor-ligand interactions between APC and the pathogen, and also by cytokines released by APC and other cells in the immediate vicinity. The released APC and cytokines are the bridge between innate and adaptive immunity. The APC “tells” the T cell what sort of defense is needed and is central to both the generation of an effective and appropriate immune response and the regulation of this response.

B lymphocytes develop in the bone marrow and emerge as naive but mature cells that circulate in the blood and lymph and lurk in the secondary lymphoid organs waiting to encounter antigen. Each mature B cell bears a unique membrane-bound B cell receptor, an immunoglobulin, or antibody molecule that is specific for a discrete motif or epitope on an antigen. Once the naive B cell encounters antigen and is activated, it undertakes a tightly regulated proliferation and differentiation program in which antigen-specific memory B cells and effector plasma cells are generated. The plasma cells secrete large amounts of antibodies, which are soluble but otherwise identical versions of the membrane-bound B cell receptor. In the first encounter with antigen, a primary antibody response is generated, later, a reencounter with the same antigen causes a more rapid secondary response, producing high levels of antibodies with a high binding affinity for the target antigen. It is this process that is exploited in prophylactic vaccination.

Unlike T lymphocytes, B lymphocytes can recognize antigen in the natural conformation, but only a few naive antigens can directly activate B cells and generate plasma cells. In most cases, antigen binding by the B cell receptor primes the B cell that then requires cognate help from the Th2 cell in the form of receptor-ligand interactions and cytokines to go through the differentiation program. T cell help is crucial for class switching, generating different antibody classes and isotypes, and developing antigen-specific memory B cells [3].

2. Host defense to human papillomavirus (HPV) infections

2.1. The infectious cycle

The papillomaviruses are ubiquitous infectious agents that are characterized by strict species specificity and tissue tropism. The infectious cycle of these viruses is tailored to the differentiation program of the target cell. Different phases of permissive viral growth accompany the maturation of the keratinocyte as it progresses up the epithelium to become a terminally differentiated squame (Fig. 1). Infection and vegetative viral growth are absolutely dependent upon a complete program of keratinocyte differentiation. The virus infects primitive basal keratinocytes, probably targeting stem cells, but only expresses high levels of viral proteins and viral assembly in the upper layers of the stratum spinosum and granulosum of the squamous epithelia [4–6]. Clinical evidence indicates that viral gene expression is confined to the keratinocyte, or cells with the potential for squamous maturation. The time from infection to release of virus is approximately 3 weeks, the time required for the basal keratinocyte to undergo com-
IL-12, TNF-

milieu is dominated by proinflammatory cytokines such as treating lymphocytes express activation markers, the cytokine and macrophages in the wart stroma and epithelium. The infil-

tation reveals a large infiltrate of T cells (both CD4+ and CD8+ stroma. Histologic examination of regressing genital warts

cells, and mononuclear cells are present mainly in the site of infection; the few intraepithelial lymphocytes are

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The infected keratinocyte then enters the differentiating compartment of the epithelium, exiting the cell cycle. Virus gene expression is hugely upregu-
lated with viral DNA amplification generating thousands of viral genomes. Late viral proteins L1, L2, and E4 are made; and virus assembly occurs in
the superficial terminally differentiated squames.

complete differentiation and desquamation. The period between infection and the appearance of lesions is highly variable and can vary from weeks to months [7], suggesting that the virus can effectively evade the immune system. There is no cytol-
ysis or cytopathic death as a consequence of HPV replication, assembly, or viral particle release because the keratinocyte is a cell destined for death and desquamation, far from the sites of immune surveillance. To permit virus replication, viral proteins actually delay nuclear condensation in the differen-
tiating keratinocytes forming the koilocyte. The virus-laden keratinocyte then dies of “natural causes.” Thus, HPV infec-
tion is not accompanied by inflammation, and there is no obvi-
ous “danger signal” to alert the immune system to the virus’s presence. This may result in persistent, chronic infection, as the host can remain ignorant of the pathogen for long periods.

2.2. Cell-mediated immunity

Clues to the nature of the cellular immune response to HPV infection have come from immunohistologic studies of spontaneously regressing genital warts. Nonregressing gen-

eral warts are characterized by a lack of immune cells at the site of infection; the few intraepithelial lymphocytes are CD8 cells, and mononuclear cells are present mainly in the stroma. Histologic examination of regressing genital warts reveals a large infiltrate of T cells (both CD4+ and CD8+) and macrophages in the wart stroma and epithelium. The infiltr-
tating lymphocytes express activation markers, the cytokine milieu is dominated by proinflammatory cytokines such as IL-12, TNF-α, and IFN-γ, and there is upregulation of the adhesion molecules required for lymphocyte trafficking on

the endothelium of the wart capillaries [8]. This is character-

istic of a Th1-based immune response, but it is important to remember that cross-sectional studies provide only a snap-
shot of a dynamic process. Ethical and logistical issues inhibit detailed longitudinal studies in humans, but in animal mod-
els of mucosal papillomavirus infection, such as the canine oral papillomavirus, the immunologic events of the entire wart cycle, from infection to regression, can be followed. In these animal infections, wart regression is accompanied by a cellular infiltrate similar to that seen in regressing geni-
tal warts. Systemic T cell responses directed towards HPV early (E) proteins E2 and E6 peptides can be detected at low frequency at distinct time points during the infectious cycle. These responses occur in narrow time windows that coincide with periods of viral DNA amplification, are maximal at wart regression, and decline quite rapidly thereafter (Jain et al., 2003, personal communication). Furthermore, serum levels of neutralizing antibody peak at wart regression [9]. Despite the low antibody titers induced by natural infection, the animals remain resistant to challenge with large doses of infectious virus for the rest of their lives.

Epidemiologic and natural history studies strongly suggest that the human immune response to HPV infection (Fig. 2) follows a similar pattern [10,11]. Virtually all studies show that genital HPV infection is extremely common in young sexually active women, with prevalence as high as 80% in certain adolescent populations [14]. Most of these HPV infec-
tions “clear,” i.e., DNA for a specific HPV type can no longer be detected. The time required for clearance of the high-risk HPV types, particularly HPV 16, averages 8–14 months, con-
siderably longer than the 5–6 months needed for the low-risk HPV types [12–14]. However, if the immune response fails to clear or control the infection, then a persistent infection, often with focally high levels of high-risk HPV DNA replica-
tion, is established. Persistently infected individuals have an increased probability of progression to high-grade cervical intraepithelial neoplasia and invasive carcinoma [10,15–18].

The increased incidence and progression of HPV infec-
tions in immunosuppressed individuals illustrates the crit-
ical importance of cell-mediated immune responses in the resolution and control of HPV infections. Patients infected

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tating lymphocytes express activation markers, the cytokine milieu is dominated by proinflammatory cytokines such as IL-12, TNF-α, and IFN-γ, and there is upregulation of the adhesion molecules required for lymphocyte trafficking on
with human immunodeficiency virus (HIV) show multiple recurrences of cervical intraepithelial neoplasia [19] and an increased incidence of genital warts [20], the latter of which appears to reflect an increased risk of progression from sub-clinical to clinical HPV infection [21]. Prospective studies show prolonged persistence of HPV DNA in HIV-infected 13- to 18-year-old girls who are otherwise healthy [22], and report high incidence rates of high-grade squamous intraepithelial lesions in this group [23]. The risk for incident high-grade squamous intraepithelial lesions in these HIV-infected girls appeared to be due primarily to the persistence of low-grade lesions, rather than to the persistence of high-risk HPV DNA without a detectable lesion [23], implying that florid viral gene expression in a persistent, active infection is important in disease progression.

2.3. Immune evasion mechanisms

Why the immune system ignores or fails to detect HPV infection for so long is a central question. HPV infections are exclusively intraepithelial and, theoretically, HPV attack should be detected by the APC of squamous epithelia, the Langerhans cell (LC). The activated LC should then migrate to the draining lymph node, processing HPV antigens en route, and present antigen to naive T cells in the node. The T cells should then differentiate into armed effector cells, migrate back to the infected site, and destroy the infected keratinocytes (Fig. 3).

There are several reasons why this does not happen. The infectious cycle of HPV is itself an immune evasion mechanism inhibiting host detection of virus. HPV replication and release do not cause cell death, since the differentiating keratinocyte is already programmed to die, and this “death by natural causes” does not present as a danger signal to the immune system. Indeed, cell death that can generate danger signals is a prerequisite for inflammation. Thus, for most of the HPV infectious cycle, there is little or no release of the proinflammatory cytokines important for dendritic cell activation and migration into the local milieu, and the essential signals required for immune responses in squamous epithelia are absent [24].

However, even in the absence of viral-induced cytolysis and cell death, HPV-infected keratinocytes should activate the powerful antiviral defense system, type I interferon secretion. The type I interferons, IFN-α and IFN-β, have antiviral, antiproliferative, antiangiogenic, and immunostimulatory properties, act as a bridge between innate and adaptive immunity, and activate immature dendritic cells [25,26]. Most DNA viruses have evolved mechanisms for inhibiting IFN synthesis and signaling, and the papillomaviruses are no exception. High-risk HPV viruses downregulate IFN-α-inducible gene expression [27,28], and the HPV 16 E6 and E7 oncoproteins directly interact with components of the interferon signaling pathways [29,30], abrogating these pathways. Capsid entry is usually an activating signal for dendritic cells, but there is evidence that LCs, unlike stromal dendritic cells, are not activated by uptake of HPV capsids [31,32], a phenomenon that would inhibit both LC migration and maturation, and the priming of the immune response against the capsid proteins. In summary, the scenario that emerges from this is as follows: HPV efficiently evades the innate immune response and delays the activation of the adaptive immune response. The host dendritic cells are exposed to low levels of viral proteins in a noninflammatory milieu for a protracted time period and, as a result, local immune nonresponsiveness may be established in the infected mucosa [33]. In this operationally HPV antigen-tolerant milieu, host defenses become irrevocably compromised, and HPV antigen-specific effector cells are either not recruited to the infected area, or their activity is downregulated, or both. Thus, if during a persistent HPV infection there is deregulation of high-risk HPV E6 and E7 with increased protein expression, and this does not result in an armed effector cell-mediated immune response, HPV-mediated progression to high-grade squamous intraepithelial lesions and invasive carcinoma are unimpeded.

3. Immune intervention in HPV infections

Despite HPV’s ability to impede host defenses, a successful immune response to genital HPV infections is established in most cases. This seems to be characterized by strong, local, cell-mediated immunity that is associated with lesion...
regression and the generation of serum neutralizing antibody. Such antibody is generated in most, but not all, infected individuals [34–36], and is directed against conformational epitope(s) on the L1 protein displayed on the outer surface of the intact virus particle. Serum neutralizing antibody levels following natural HPV infections, even at peak titers, are low [37]. This probably reflects the exclusively intranuclear infectious cycle (the absence of a viremia), as well as the production of virus particles in the superficial epithelial cells, distant from APCs and patrolling macrophages. These factors limit antigen uptake, delivery to the lymph node, and presentation to naïve B and T cells. Despite these low antibody levels, seropositive animals are protected against further viral challenge [38], and this protection can be transferred from resistant to naïve animals by passive transfer of serum [39].

This suggests that a vaccine that generates neutralizing antibody to the major capsid protein L1 of genital HPVs would be protective against infection. L1 protein must be in the tertiary or native form and assembled as a multimer for neutralizing antibody to be generated [40], a technically difficult objective. Production of L1 proteins has been achieved by inserting the L1 gene into expression vectors, such as recombinant baculoviruses for expression in insect cells, or plasmids for expression yeast. In these cells, exogenously expressed L1 proteins self-assemble into L1 virus-like particles (VLPs) [41,42]. The L1 VLP is a conformationally correct, empty (containing no DNA) capsid that appears morphologically identical to, and contains the major neutralizing epitopes of, the native HPV virion [43,44].

In experimental studies using the dog, cow, and rabbit, immunization with L1 VLPs induced circulating neutralizing antibody to the L1 capsid protein, and the animals were completely resistant to challenge with large amounts of virus (for review, see [45]). This made L1 VLPs clear candidate immunogens for prophylactic vaccinations in humans. Dose-ranging phase I studies in healthy subjects showed that doses of 9–100 μg HPV L1 VLPs given in three injections over 4–6 months were highly immunogenic, generating high titers of anti-L1 antibody. All VLP-immunized subjects, but no subjects in the placebo arms, seroconverted and demonstrated anti-VLP antibody responses that were substantially greater than those identified following natural infections [46–49]. The dominant antibody responses induced by VLP vaccines are of the IgG1 subclass and have been shown to be neutralizing by a variety of surrogate neutralization assays [46,50,51].

### 3.1. Type specificity and cross-protection

The neutralizing antibodies generated by HPV L1 VLP vaccines appear to be type specific [52,53]. Thus, immunization with HPV 16 L1 VLPs would be expected to protect against HPV 16 infection but not against any of the other genital HPV types. The current generation of VLP vaccines contains only two of the high-risk HPV types, HPV 16 and 18. HPV 16 accounts for 50–60%, and HPV 18 10–12%, of cervical cancer cases; thus, in the best-case scenario with 100% vaccine coverage of the target population, approximately 70% of cervical cancers would be prevented unless vaccines induce significant cross-protection against other oncogenic HPV types. If such cross-protection does in fact occur, then the mechanism is unclear, since experimental evidence shows that the neutralizing antibodies generated by VLPs are type specific. The only known HPVs that share a neutralizing epitope are HPV 6 and 11 [44], HPV 31 and 33, and HPV 18 and 45 [54]. Type-common neutralizing linear epitopes do exist, but the cross-neutralization induced by them may be too low to be protective [55]. VLPs have been shown experimentally to induce strong innate [56,57] and cell-mediated responses [58], T cell responses to VLPs have been measured by lymphoproliferation and cytokine assays [59,60]. In one study some cross-reactivity to HPV 16 VLPs was observed [47], implying that T helper epitopes are conserved across serologically distinct genotypes. However, this has not been observed consistently, and whether T cell cross-reactivity to HPV L1 is general and translates into cross-protection remains to be demonstrated.

#### 3.2. Vaccines in clinical trials

Two L1 VLP vaccines are now in phase III trials: a bivalent HPV 16/18 VLP vaccine and a quadrivalent HPV 6/11/16/18 vaccine. Recent double-blind, placebo-controlled trials of adjuvant HPV 16/18 L1 [61], or HPV 6/11/16/18 L1 VLPs [37] have yielded encouraging efficacy data. VLP vaccination with HPV 16/18 L1 VLPs in women testing negative for HPV 16/18 is safe and protective, preventing both persistent HPV 16/18 infection and the development of low-grade intraepithelial lesions [61]; this vaccine is not effective at preventing genital warts, since it does not provide protection against any low-risk HPV types.

In the efficacy trial for the quadrivalent vaccine, all vaccine doses produced similar levels of antibody to each VLP component of the vaccine. In this study, there were no cases of cervical intraepithelial neoplasia or external genital warts in the vaccinated group, compared to seven and four cases, respectively, in the placebo group [37]. This confirms the results of previous trials showing that HPV 16 or HPV 16/18 vaccines confer protection against cervical intraepithelial neoplasia induced by these respective HPV types [61,62], and strongly suggests that anogenital disease induced by HPV 6 or 11 can be controlled. The benefits of reducing both benign and malignant HPV-associated genital disease are immense, both economically, in terms of reduced health care costs, and even more important, with respect to the improvements in human health and well-being that would result.

### 4. Summary

HPV infection of the genital tract is common in young sexually active individuals, the majority of whom clear the infection without overt clinical disease. Most of those who
develop benign lesions eventually mount an effective cell-mediated immune response that results in lesion regression. Regression of anogenital warts is accompanied histologically by a CD4+ T-cell-mediated Th1 response, and data from animal models suggest that the response is modulated by CD4+ T-cell-dependent mechanisms. Failure to develop effective cell-mediated immunity to clear or control infection results in persistent infection and, in the case of the high-risk HPVs, an increased probability of progression to high-grade squamous intraepithelial lesions or invasive carcinoma. The mechanism that prevents cure of oral papillomavirus type 31 MID grade lesions in individuals immunosuppressed as a consequence of HIV infection demonstrates the importance of CD4+ T cells in the control of HPV infection. The prolonged duration of infection associated with HPV seems to be associated with effective evasion of innate immunity as reflected in the absence of inflammation during virus replication, assembly, and release, and downregulation of interferon secretion and response, thus delaying the activation of adaptive immunity. Serum neutralizing antibody to the major capsid protein L1 is usually produced after successful induction of cell-mediated immunity, and these antibody and cell-mediated responses protect against subsequent viral challenge in natural infections in animals. Prophylactic immunization using L1 VLPs has proven effective in all animal models tested, and phase 2 results in humans suggest that HPV VLP vaccines are safe, immunogenic, and efficacious; these vaccines are expected to yield significant public health benefits.

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