

Immunobiology of HPV and HPV vaccines

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Abstract

Genital human papillomavirus (HPV) infection with both low- and high-risk types is common, but most infections resolve as a result of a cell-mediated immune response. Failure to induce an effective immune response is related to inefficient activation of innate immunity and ineffective priming of the adaptive immune response; this defective immune response facilitates viral persistence, a key feature of high-risk HPV infection. This milieu becomes operationally HPV antigen tolerant, and the host's defenses become irrevocably compromised. HPV antigen-specific effector cells are poorly recruited to the infected focus and their activity is downregulated; neoplastic HPV containing cervical keratinocytes expressing high levels of E6 and E7 oncoproteins are not killed in this immunosuppressive, tolerant milieu, and progression to high-grade disease and cancer can result. Highly efficacious prophylactic HPV L1 virus-like particle (VLP) vaccines circumvent viral epithelial evasion strategies since they are delivered by intramuscular injection. The stromal dendritic cells of the muscle that encounter the highly immunogenic repeat structure of the VLP then migrate with their cargo to the lymph node, initiating an immune cascade that results in a robust T-cell dependent B-cell response, which generates high levels of L1-specific serum neutralizing antibodies and immune memory.

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Introduction

It is sobering to reflect that as recently as 1970 it was assumed that there was only one human papillomavirus (HPV) and that it was the cause of various warty lesions that decorated different tissue sites. HPV was seen, except in rare instances [1], as causing unsightly but essentially trivial excrescences that, given time, would regress spontaneously. The advent of recombinant DNA technology overturned this simple view of the HPV world, and it became clear within a decade that there were multiple HPVs and that the warts on different tissue sites were caused by different HPV types with a tropism for mucosal or cutaneous squamous surfaces [2]. It also became evident that HPV did not cause the trivial disease but that some members of the HPV family, particularly a subset infecting the anogenital

tract, were true human carcinogens and were the cause of carcinoma of the cervix, the second most common cancer in women worldwide.

HPVs are a very large family comprising (at present) more than 130 genotypes that have been cloned from various clinical lesions. These viruses are not classified as serotypes but as genotypes on the basis of DNA sequence [3]; because in vitro culture of these viruses is problematic, HPV infection is determined by detecting HPV-DNA. The viruses have a predilection for either cutaneous or mucosal epithelial surfaces and fall into two groups — low-risk types that predominantly cause benign warts or high-risk types associated with malignant disease. This risk stratification is shown clearly in the genital tract where about 30 to 40 HPVs regularly or sporadically infect the mucosal epithelium of men and women. The two most common low-risk viruses that cause warts on the anogenital mucosae are HPV 6 and 11. There are about 15 oncogenic or high-risk HPVs that infect the genital tract, but the two major players are HPV 16 and

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18, which are responsible for 70% of cervical carcinomas worldwide [4].

The HPV infectious cycle — an immune evasion strategy

From the evolutionary biological standpoint, HPVs are very successful infectious agents — they induce chronic infections that have no systemic sequelae and rarely kill the host but periodically shed large amounts of infectious virus for transmission to naive individuals. To achieve this lifestyle, HPV must avoid the host defense systems; the key to understanding how this is achieved is the virus replication cycle (Fig. 1), which in itself, is an immune evasion mechanism that inhibits the host's detection of virus. Infection and vegetative HPV growth are absolutely dependent upon a complete program of keratinocyte differentiation. Virus infects primitive basal keratinocytes, probably targeting stem cells, but high level viral expression of viral proteins and viral assembly occur only in the upper layers of the stratum spinosum and granulosum of squamous epithelia [5]. Viral gene expression is confined to the keratinocyte; there is no evidence that viral genes are expressed in any cell other than keratinocytes, and there is a spatial and temporal pattern of HPV gene expression in the infected epithelium. The virus infects a subset of primitive basal cells, probably stem cells, at low copy number. Sometime after infection, there is a round of viral DNA replication that appears to be independent of the cell cycle and amplifies the viral copy number to around 50 to 100 copies per cell. The infected cell is thought to then leave this primitive stem cell-like compartment and enter the transit amplifying, proliferative compartment of the epithelium. There is then a phase of plasmid or episomal maintenance when viral gene expression is minimal; specifically, the expression of oncogenes E6 and E7 is under very tight control, with E6/E7 transcripts barely detectable. When the infected keratinocyte enters the differentiating compartment, exiting the cell cycle, there is a massive upregulation of viral gene expression and viral DNA replication occurs; there is amplification of viral copy number to at least 1,000 copies per cell, abundant expression of the early genes E6 and E7, and expression of the late genes from the late promoter [6].

The infectious cycle of high-risk HPV is a high-risk strategy for the host

E6 and E7 expression is kept under strict control

It is important to recognize that these events occur in cells that are differentiating and have exited the cell cycle. Papilloma-viruses encode only one DNA replication enzyme, E1, and apart from this and the viral E2 protein, replication is totally dependent upon the cellular DNA synthetic machinery. The challenge for the virus is that the cellular DNA polymerases and replication factors are only produced in mitotically active cells. To solve this problem, the viruses encode proteins that, in the context of the viral life cycle, reactivate cellular DNA synthesis in non-cycling cells, inhibit apoptosis, and delay the differentiation program of the infected keratinocyte, creating an environment permissive for viral DNA replication. The precise details by which this is achieved are imperfectly understood, but the viral genes central to these functions are E6 and E7. Unfortunate but rare by-products of this role in high-risk HPV replication are the deregulation of growth control in the infected cell and the development of cancer [7].

Immune ignorance and HPV

In this infectious cycle, the virus is basically a hitchhiker joining the keratinocyte at the start of its journey as a primitive basal cell in the epithelium through to its end as a terminally differentiated squame. It is a replication strategy in which viral DNA replication and virus assembly occur in a cell that will terminally differentiate and die by natural causes. Thus, there is no viral-induced cytolysis or necrosis, and therefore no inflammation. For most of the duration of the HPV infectious cycle, there is little or no release into the local milieu of pro-inflammatory cytokines, which is important for antigen presenting cell (APC) activation and migration. The central signals to kick start the immune responses in squamous epithelia are absent [8]. There is no blood-borne or viremic phase of the HPV life cycle, and only minimal amounts of replicating virus are exposed to immune defenses; in effect, the virus is practically invisible to the host who remains ignorant of the pathogen for long periods of time.

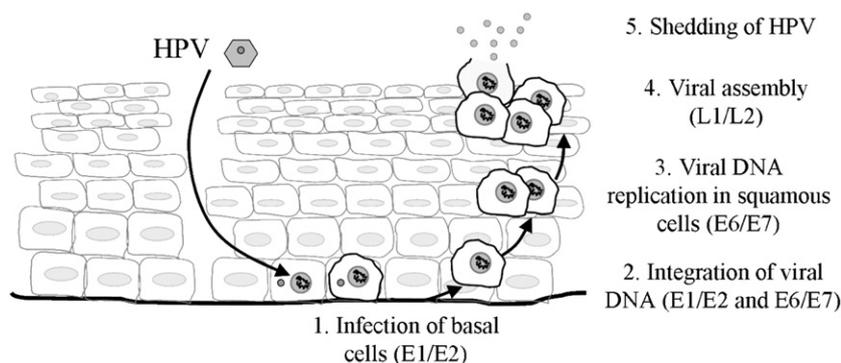


Fig. 1. Life cycle of human papillomavirus.

High-risk HPVs disable Langerhans cells

HPV infections are exclusively intraepithelial. Theoretically, HPV attacks should be detected by the professional APC of squamous epithelia, the Langerhans cell (LC), which is the intraepithelial dendritic cell (DC). Virus capsid entry is usually an activating signal for DCs, but there is evidence that LCs are not activated by the uptake of HPV capsids. LCs, when incubated with L1 virus-like particles (VLPs) of HPV 16, do not initiate epitope-specific immune responses against L1 derived antigens and, in effect, are tolerized by VLP uptake [9]. In contrast, stromal DCs are activated by VLPs and stimulate HPV-specific T-cells [10], but since the virus remains in the epithelium the probability of encountering stromal DCs is low, effectively disabling a key component of the immune response.

High-risk HPVs downregulate interferon gene responses

Even in the absence of viral-induced cytolysis and cell death, HPV infected keratinocytes should activate the production of type 1 interferons, a powerful, generic, antiviral, and innate immune defense system. The type 1 interferons, IFN- α and IFN- β , have antiviral, antiproliferative, antiangiogenic, and immunostimulatory properties that act as a bridge between innate and adaptive immunity, activating immature DCs [11]. Most DNA viruses have mechanisms for inhibiting interferon synthesis and receptor signaling, and papillomaviruses are no exception. High-risk HPV infection downregulates IFN- α inducible gene expression and the HPV 16 E6 and E7 oncoproteins directly interact with components of the interferon signaling pathways (reviewed by Kanodia and Kast) [12]. DNA microarray analysis of gene expression shows that HPV 16 E6 and E7 alter expression of interferon response genes, NF kappa B stimulated genes, and cell cycle regulation genes [13,14], and therefore directly alter the expression of genes that enable host resistance to infection and immune function.

Immune response to HPV in natural infections

Despite the best efforts of the virus to evade host defenses, most HPV infections resolve with time. Anogenital warts and low-grade intraepithelial lesions are cleared as a result of a successful cell-mediated immune response [15] directed against early HPV proteins, particularly E2 and E6 [16,17]. In animal infections, this cell-mediated response [18] is closely followed by seroconversion and antibodies to the major coat protein, L1 [19]. This is probably also true in humans [8]; however, the antibody concentrations achieved in animals and humans are low, and many women do not seroconvert [20–22]. This observation should be tempered by the recognition that the current methods of measuring antibody concentration are relatively insensitive with a low signal to noise ratio. There is no viremia in natural infections. Furthermore, free virus particles are shed from the surface of squamous epithelia with poor access to vascular and lymphatic channels and to lymph nodes where immune responses are initiated (Fig. 2).

Although 80% to 90% of genital HPV infections resolve with time, about 10% to 20% of individuals do not become HPV-DNA negative and develop persistent infection. This group is at high risk for progression to high-grade cervical intraepithelial disease, cervical intraepithelial neoplasia (CIN) 2/3, a condition characterized (in biological terms) by the expression of HPV E6 and E7 proteins in dividing cells, chromosomal instability, and the progressive ability to resist both innate and adaptive antiviral immune defenses. Integration of HPV-DNA into the host chromosome is a well recognized event that has occurred in a high proportion of cervical carcinomas [23]; episomal HPV is cleared from cells after exposure to IFN- β , but cells with integrated HPV-DNA are resistant to this antiviral effect [24,25]. T-cell responses to E2 and E6 are lost or reduced in CIN 3 and invasive carcinoma [26]. Even if HPV antigen-specific cytotoxic T-cells have been generated, regulatory T-cells increasingly dominate the lesions and abrogate the killer defense response [27]. The challenge for therapeutic vaccines for HPV associated

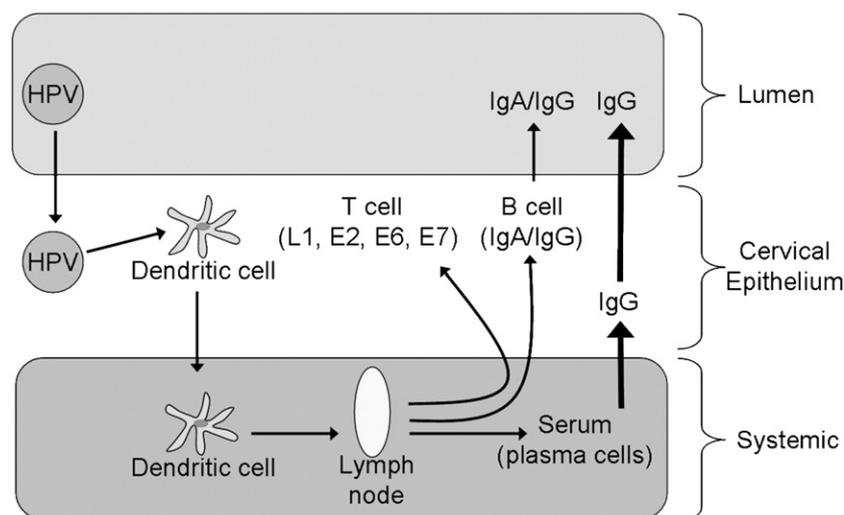


Fig. 2. Simplified summary of immunological response to HPV infection.

disease is to reverse this immunologically suppressive micro-environment and allow the cytotoxic killers to access the infected and neoplastic cells — a task that has remained elusive to date.

Prophylactic HPV vaccines

So why, if natural antibody responses are so poor, should vaccines that generate serum neutralizing antibodies protect? The evidence from animal papillomavirus infections, including some of the earliest published works from Shope, the founding father of papillomavirus research, showed very clearly that neutralizing antibodies were protective [28]. In Shope's experiments, if rabbits were infected systemically with the cotton tail rabbit papillomavirus (CRPV) by direct injection of virus into the muscle or bloodstream, papillomas did not arise on the skin of the challenged animals and neutralizing antibodies were generated; the animals were completely resistant to subsequent viral challenge by abrasion of the epithelium. This and other data suggested very strongly that generating neutralizing antibodies to virus capsid proteins would be an effective prophylactic vaccine strategy and this has proved to be so. HPV L1 VLP vaccines induce high concentrations of neutralizing antibodies to L1, and virtually all subjects in the vaccine trials have seroconverted [29;30]. HPV VLP vaccines are delivered intramuscularly, resulting in rapid access to the local lymph nodes, thus circumventing the immune avoidance strategies of the viral intraepithelial infectious cycle. Furthermore, VLPs are highly immunogenic, inducing potent antibody responses in the absence of adjuvant [31] due to their ability to activate both innate and adaptive immune responses. VLPs are rapidly bound by myeloid DCs and B lymphocytes, and signal via the toll-like receptor (TLR) dependent pathway MYD88 [32;33], which is essential for B-cell activation and antibody generation in mice and probably in humans.

Immunogenicity and mechanism of protection

Prophylactic HPV vaccines have been shown to be highly efficacious in the various Phase 2 and Phase 3 randomized control trials (RCTs) [34;35;36] (reviewed by Koutsky and Harper) [37]. However, only data from the first 5 to 6.4 years of these trials are published [30;38], and since these vaccines will need to provide protection over decades, there are some key questions to address:

- Are there immune correlates that predict protection?
- What is the mechanism of this protection?
- What is the likely duration of protection?

Mechanism of protection

At present, there is no immune correlate of protection. Virtually all vaccinated individuals have seroconverted; the peak geometric mean antibody concentrations achieved are at least two logs higher than those after natural seroconversion. Currently, the best assumption is that the mechanism of

protection elicited by VLPs is serum antibodies. The most unequivocal evidence for this notion comes from experiments in rabbits [39] and dogs [40,41], in which it was shown that naive animals passively immunized with purified serum IgG from either VLP immunized or naturally infected animals were completely protected against high viral challenge. The mechanism by which neutralizing antibodies to HPV prevent viral entry is speculative at present. However, there are new data on how the virus enters the keratinocyte, which suggest different stages at which neutralizing antibodies could be effective. Recent studies have shown that HPV infection requires a micro-abrasion of the squamous epithelium that results in epithelial denudation but retention of the epithelial basement membrane [42]. HPV initially binds by a primary receptor to this exposed basement membrane before entering the keratinocyte, presumably as the keratinocyte migrates along the basement membrane to repair the small wound. This is a protracted process extending over 24 to 48 h, during which the virus capsid undergoes conformational changes. It is speculated that such changes expose the secondary receptor by which the virus binds to and enters the keratinocyte (Day personal communication 2007). Virus neutralizing antibodies could act by binding to the receptors or by binding to the capsid and preventing the conformational distortion, which is essential for successful viral entry. Probably both types of antibodies are generated after VLP immunization, but in general, higher concentrations of blocking antibodies (anti-receptors) are needed for neutralization compared with those preventing conformational changes. It is of interest that in natural animal papillomavirus infections, for example in the dog and rabbit [43;44], low concentrations of anti L1 antibodies provide long-term protection against high doses of challenge virus.

Duration of protection

The duration of protection afforded by a new vaccine cannot be predicted at the outset of the introduction of the vaccine. The evidence from the RCTs is that protection against high-grade HPV 16 and 18, which cause intraepithelial disease, and HPV 6 and 11, which cause low-grade anogenital disease, remains at greater than 98% over a 5 to 6.4-year period [30,38]. In the majority of vaccinated subjects, serum antibody levels remain at concentrations greater than those found in natural infections over this period. However, even in those subjects that have antibody levels fall to natural infection levels or below, there is no evidence to date of vaccine breakthrough [45] but published data from RCTs extend only to 5.5 years post immunization, and the question of disease protection in the absence of detectable antibodies remains open. Most of the effective vaccines in current use rely heavily on the long-term protection of high affinity B-cell memory that develops under the guidance of helper T-cells. HPV L1 VLPs are subunit protein vaccines and Th cell-regulated evolution of B-cell memory is pivotal if long-term immune protection from subunit vaccines is to be provided. VLPs, as discussed above, are very effective at stimulating APCs and generating strong Th responses. B-cell memory is a systemic phenomenon characterized by the high titer and affinity of the antibody response when confronted with the pathogen. In general,

if primary immunization generates high titers and high affinity antibodies there is a good memory response. As might be predicted from the high antibody titers generated by the primary immunization with the prophylactic VLP vaccines, both vaccines have been shown to generate immune memory. Circulating B memory cells can be detected soon after vaccination with the bivalent vaccine [46]. Subjects vaccinated with the quadrivalent vaccine showed a classical recall response to antigens five years after the initial primary immunization [47]. Memory recall responses have been shown to be central to the protection offered by hepatitis B vaccines, and although the pathogenesis of hepatitis B and HPV are quite different, both share the phenomenon of an extended period between infection and detectable viral replication, and both induce chronic persistent infections.

Cross-protection

In natural HPV infections, the detectable neutralizing antibody responses are type-specific; however, HPV L1 VLP vaccines generate not only type-specific but cross-reactive and cross-neutralizing antibodies [48]. Both commercial prophylactic vaccines have now shown evidence of cross-protection, or protection against non-vaccine HPV types. The high antibody concentrations generated by the vaccines probably explain this phenomenon. In general, the population of antibodies produced in response to a particular antigenic stimulus such as a VLP, is heterogeneous. Most antigens are structurally complex, containing many different epitopes or antigenic determinants. The immune system responds to the antigen by producing antibodies to most of the accessible epitopes. Thus, in any response to a specific protein, there will be several populations of antibodies; the overall antibody response is polyclonal or heterogeneous, and it comprises the output of all the individual's stimulated B-cells. Epitopes recognized by B-cells are usually a confirmation and these B-cell epitopes are only displayed by proteins in the native or tertiary structure. Complex proteins such as L1 contain multiple overlapping epitopes, some of which are immunodominant — they induce a more profound and stronger response in the host than other epitopes and therefore dominate the polyclonal response. This can be seen quite clearly in the antibody response to HPV L1 VLPs. The immunodominant antibodies are type-specific antibodies but there are, of course, subpopulations of other antibodies, some of which will be to epitopes shared by other HPV types. In natural infections, the antibody concentrations generated are low so that only the immunodominant species is detected. However, in VLP immunized individuals, antibody concentrations are high and the subpopulations of cross-reactive and cross-neutralizing antibodies can therefore be detected in seroassays. Not surprisingly, there is now evidence of cross-protection afforded against these non-16 and non-18 types by L1 VLP vaccines [34]. In natural infections, antibody concentrations are low and only the immunodominant type-specific antibodies are detected using the available assay systems. However, in VLP immunized individuals, antibody concentrations are high and the immunodominant population is detected together with the subpopulations, which include cross-reactive and cross-neutralizing antibodies. These subpopulations are present at antibody

concentrations one to two logs lower than the dominant type-specific neutralizing antibodies. Not every individual will generate cross-neutralizing antibodies since immunodominance is complex and depends, among other things, upon the major histocompatibility complex haplotype of the host.

Adjuvants

HPV VLP vaccines are subunit protein vaccines, and although HPV VLPs alone are highly immunogenic [31], protein vaccines usually require adjuvants to achieve peak immunogenicity. Both commercially available VLP vaccines are formulated with adjuvants, compounds that enhance immunogenicity. The quadrivalent vaccine consists of HPV 6, 11, 16, and 18 L1 VLPs plus the proprietary adjuvant amorphous aluminum hydroxysulfate (AAHS). This proprietary adjuvant has been used in many vaccines by the manufacturer and has been delivered to millions of individuals with no evidence of toxicity. The bivalent vaccine consists of HPV 16 and 18 L1 VLPs plus the adjuvant system AS04, comprised of aluminum hydroxide and monophosphoryl lipid A (MPL), a modified endotoxin and agonist of TLR4. AS04 is one of the new generations of adjuvants. No toxicity has been reported, and AS04 has been delivered to about 90,000 individuals to date in the hepatitis B vaccine, Fendrix™, and in trials of herpes simplex virus protein vaccines (GlaxoSmithKline).

Vaccine-induced protective immunity depends largely on the activation of the appropriate antigen-specific CD4⁺Th2 cells that “help” antigen primed B-cells differentiate into antibody secreting plasma cells and memory B-cells. In natural infections, this differentiation program depends upon signals generated by the recognition of microbial products by pattern recognition receptors such as TLRs expressed on the APCs. In the vaccination setting, triggering of these signals can be achieved by adjuvants, thus activating innate immune responses that bias to an appropriate adaptive response.

In humans, aluminum salts are the most common adjuvant, inducing antibodies and Th2 type responses. The mechanism of action is imperfectly understood but it has been generally assumed that adsorption of antigen to the salt forms a depot at the vaccination site from which antigen can be released, transforming a soluble antigen to a particulate, favoring high local antigen concentrations and uptake by APCs. Aluminum hydroxide has direct effects on macrophages, activating them for antigen presentation. Recently, it has been shown that aluminum hydroxide activates caspase-1 and induces secretion of IL-18 and IL-1 β from APCs stimulated by TLR agonists [49]. These cytokines are powerful adjuvants in their own right but the combination of TLR activation and aluminum hydroxide focuses the immune response down a T-cell dependent antibody response route with the generation predominantly of serum IgG1.

Three aluminum salts are or have been used in vaccines and the different salts have different physicochemical properties. At neutral pH, aluminum hydroxide has a net negative surface charge, aluminum phosphate has a net positive charge, and AAHS is neutral. AAHS has an increased capacity to bind HPV 16 VLPs compared with other salts. In mice, AAHS adjuvanted

HPV VLPs induce antibody concentrations one half to one log higher than aluminum hydroxide adjuvanted VLPs, yet there was no significant difference in IgG titers when comparing the AAHS and aluminium phosphate adjuvants [50].

AS04 combines aluminum hydroxide and MPL, an agonist of TLR4. This combination strongly enhances the immunogenicity of VLPs compared with aluminum hydroxide alone. Thus, one month after the third immunization, subjects immunized with HPV 16 or 18 L1 VLPs plus AS04 showed antibody concentrations one half to one log higher than the aluminum hydroxide adjuvanted VLPs, and two to three times the number of circulating B memory cells [46]. AS04 represents a new generation of adjuvants that are rationally designed on the basis of understanding the immune response and combining components with different functions and activities that are synergistic.

Summary

HPVs are successful pathogens, inducing chronic infections that are exclusively local and intraepithelial, and rarely result in the death of the host or systemic sequelae. They achieve this enviable lifestyle by a combination of passive and active immune avoidance. The viral infectious cycle is confined to the epithelial compartment; there is no viremia or blood-borne spread, and virus particles are shed from mucosal surfaces far from vascular and lymphoid channels. As a result, there is poor access of virus and virus proteins to lymph nodes where adaptive immune responses are initiated. Crucially, there is no virus-induced cell death, and the inflammatory signals that would activate APCs in the epithelium are absent. Furthermore, HPVs downregulate interferon responses and disable the epithelial LCs. This allows long periods of uninterrupted virus replication in the epithelium during which the host is ignorant of virus presence. This is a high-risk strategy for the host when infection is with oncogenic genital HPVs, as it increases the risk that the host immune system may become tolerant or non-responsive to viral proteins. It also increases the risk of “accidents” in virus replication that result in the unregulated expression of viral E6 and E7 oncoproteins and neoplastic transformation.

Prophylactic HPV L1 VLP vaccines circumvent the viral epithelial evasion strategies since they are delivered by intramuscular injection. The stromal DCs of the muscle that encounter the highly immunogenic repeat structure of the VLP then migrate with their cargo to the lymph node, initiating an immune cascade that results in a robust T-cell dependent B-cell response, which generates high levels of L1-specific serum neutralizing antibodies and immune memory.

Conflict of interest statement

MS has served on the Steering Committee for GSK phase IV trial and served as a consultant for Merck Research Laboratories, Philadelphia, USA, Sanofi Pasteur MSD, Lyon, France and GSK Biologicals, Rixensart, Belgium.

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