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Development of an AS04-Adjuvanted HPV Vaccine with the Adjuvant System Approach

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Contents

Abstract

A novel human papillomavirus (HPV) vaccine has been formulated with virus-like particles of the L1 protein of HPV-16 and HPV-18, and the Adjuvant System 04 (AS04). AS04 is a combination of the toll-like receptor 4 agonist monophosphoryl lipid A (MPL) and aluminum hydroxide. The AS04-adjuvanted HPV vaccine induces a high and sustained immune response against HPV, including high levels of neutralizing antibodies at the cervical mucosa in women aged 15–55 years. Recently, the mechanism of action of AS04 has been evaluated *in vitro* in human cells and *in vivo* in mice and the data provide evidence for the molecular and cellular basis of the observed immunogenicity, efficacy, and safety profile of this formulation.

In this review, we discuss how the results of GlaxoSmithKline's clinical studies on immunogenicity, protection, and reactogenicity with the AS04-adjuvanted HPV vaccine are supported by the observed mechanism of action for the adjuvant.

The adjuvant activity of AS04, as measured by enhanced antibody response to HPV antigens, was found to be strictly dependent on AS04 and the HPV antigens being injected at the same intramuscular site within 24 hours of each other. The addition of MPL to aluminum salt enhances humoral and cell-mediated response by rapidly triggering a local and transient cytokine response that leads to an increased activation of antigen-presenting cells and results in an improved presentation of antigen to CD4+ T cells.

The added value of MPL in AS04 for an HPV vaccine was demonstrated in clinical studies by high vaccine-elicited antibody responses and the induction of high levels of memory B cells. The vaccine elicits cross protection against some other oncogenic HPV types (specifically HPV-31, -33, and -45) not contained in the vaccine. The localized and transient nature of the innate immune response supports the acceptable safety profile observed in clinical studies.

Vaccination represents one of the most successful achievements in public health to date, having reduced the burden of infectious disease for millions of people worldwide. However, vaccinology is still evolving rapidly in response to continuously developing challenges presented by both pathogens and the immune status of some subjects. Recent advances in immunobiology have led to an important shift in the way vaccine design is approached. One major focus today in this regard is the enhancement of immune responses by pairing purified antigens with combinations of appropriate adjuvants.

This review describes the formulation, selection, and mechanism of action of the immunostimulating Adjuvant System 04 (AS04), a combination of monophosphoryl lipid A (MPL) and aluminum salts. AS04 will be discussed in the context of the novel human papillomavirus (HPV) vaccine containing antigens from the two types of HPV most frequently found in cervical cancer: HPV-16 and -18. The clinical profile of the vaccine will also be discussed in light of the recently described innate immunity events induced after AS04 injection.[1]

1. From an Empirical to a More Targeted Approach to Vaccine Design

From the time of Jenner's first cowpox inoculations, vaccination has generally proven to be very cost effective. It has resulted in the eradication of smallpox and removal or minimization of the threat of polio, diphtheria, tetanus, measles, and many other diseases.[2] Historically, vaccines have been developed empirically with formulations of either inactivated whole pathogens or live attenuated organisms with limited virulence. However, it has become evident that not all vaccines can be developed using the method of inactivation or live attenuation, since some vaccines based on whole pathogens remain too reactogenic even after inactivation or because some other pathogens can not be attenuated in a suitable way for use in a vaccine.[3] To overcome those issues, other methods were introduced, such as antigen purification or production of recombinant proteins in expression systems.

The ability to select and purify subunits or single protein antigens has led to improved vaccine tolerability whilst maintaining the necessary antigen-specific components. However, in most cases the purification process resulted in lower immunogenicity of the antigen. It is indeed known that the purification process alters or removes the natural microbial entities that help trigger the immune response.[4]

The immune response to pathogens relies on two important components: the antigen for specific recognition of the pathogen by T and B lymphocytes, and other structural elements of the pathogen that provide an alert signal to induce adequate activation of the innate immune system.[5]

In vaccines, the addition of adjuvants compensates for the loss of natural microbial immune triggers caused by inactivation and/or purification processes. An adjuvant may be defined as a substance or compound that is able to improve the quality and magnitude of an immune response through the stimulation of innate immunity and, in particular, antigen-presenting cells (APCs). They may be purified from pathogens or derived from other natural or synthetic sources.

Aluminum salts have been used as adjuvants in human vaccines for over 70 years.[6,7] This approach is still useful for vaccines where antibodies represent the main protection mechanism, since aluminum salts have been proven to enhance humoral immune response. However, aluminum salts are often not sufficient when higher levels of antibodies and/or T-cell-mediated immunity, especially T helper-1 (T_h1)-biased responses, are required.[6] Approximately 2 decades ago, various research efforts were initiated to identify new adjuvants capable of meeting these needs.[8-10] In the mid 1990s, oil-in-water emulsions such as squalene-derived adjuvants and virosomes, including liposomes and influenza glycoproteins, were developed for use in vaccines.[11-14] In particular, when combined with antigen, the squalene-based oil-in-water emulsion MF59 demonstrated the ability to induce increased T- and B-cell response compared with aluminum salts.[15] In parallel, it was discovered that adjuvants could be combined to provide the individual benefits of their components in a single formulation.[12] The GlaxoSmithKline Adjuvant System (AS) family, including AS01, AS03, and AS04, are examples of this new concept in vaccine design and have demonstrated their ability to promote strong humoral and cellular immune responses, showing proof of concepts for several diseases.[12,16-18]

1.1 New Vaccines for Unmet Medical Needs

Effective vaccines for diseases caused by complex parasites with multistage life cycles such as malaria, viruses suppressing intrinsic host immunity or undergoing rapid mutations such as HIV, or intracellular pathogens such as *Mycobacterium tuberculosis*,[19,20] remain thus far out of reach. One way to develop potent vaccines for these diseases is to carefully select the antigenic component and adjuvant in order to promote a robust and sustained pathogen-specific B- and T-cell immunity.

Beside protection against challenging pathogens, a better knowledge of the immune status of certain populations is also a key factor in the development of a vaccine. Very young children with immature immune systems, elderly individuals with

immunosenescence, and immunosuppressed and chronically ill patients represent key populations to consider. They can elicit a weak immune response to classical vaccines and may, therefore, not achieve the same level of protection against infections or diseases as healthy individuals.[21-23] Clinical results with different vaccines based on the approach of new adjuvants and adjuvant combination have shown that appropriate adjuvantation can help to overcome the challenges as described previously.

To understand how the careful selection of adjuvants and antigen can impact vaccine efficacy, it is important to refer to key concepts of modern immunology.

1.2 Link between Innate and Adaptive Immunity

The human immune system comprises both innate and adaptive immune systems, which are closely linked together (figure 1). Innate immunity represents a first line of host defense against pathogens that enter the body (figure 1a). Innate immunity defense mechanisms are mediated by both stromal and immune resident cells, and recruited immune cells such as monocytes and granulocytes. These defense mechanisms operate rapidly after infection but lack specificity and memory. Adaptive immunity provides a second line of defense and is characterized by the amplification of antigen-specific T and B cells (figure 1b and figure 1c). The amplification leads to the production of circulating antibodies and effector T cells that are able to sense and destroy infected/pathogenic cells (figure 1c). Moreover, adaptive immunity is able to establish immune memory, providing a stronger and faster immune reaction when challenged by the same pathogen again.

The response of T and B lymphocytes to a pathogen or vaccine is largely directed by the initial encounter with the host and the activation of innate immune cells through specific innate receptors that recognize conserved motifs expressed by microbes (figure 1a). The best characterized of these are the toll-like receptors (TLRs) that recognize well described molecular motifs common to either viruses, bacteria, or parasites.[24,25] The recognition of these molecular patterns stimulates the host innate sentinel cells (macrophages and epithelial cells) to secrete cytokines/chemokines. These chemoattractants can in turn recruit and activate

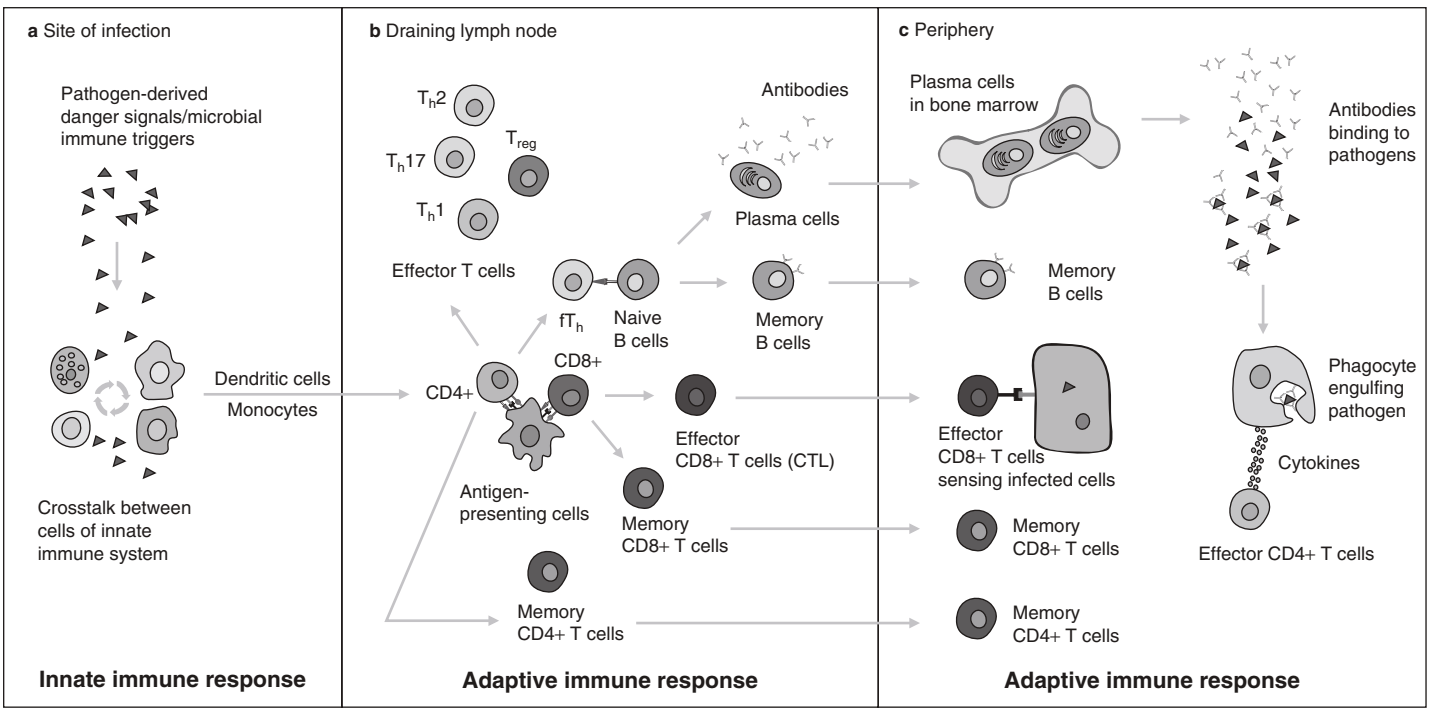

Fig. 1. Main steps in innate and adaptive immune responses. (**a**) Pathogen-derived danger signals or adjuvants activate the innate immune response and trigger maturation of dendritic cells (resident or derived from recruited monocytes) and their migration to lymph nodes. (**b**) Antigen-presenting cells present antigen to naive T cells in lymph nodes, and depending on the nature of co-stimulating signals and secreted cytokines, the differentiation of naive T cells to different effector and memory CD4+ and CD8+ T cells is initiated. The most common pathways for CD4+ cells are differentiation into T helper-1 (T_h1), T_h2, and T_h17 cells, follicular helper (fT_h) cells, or regulatory T (T_{reg}) cells. The CD4+ fT_h cells provide signals for B-cell differentiation into antibody-producing plasma cells and memory B cells. CD8+ cells can differentiate into cytotoxic T cells and/or cytokine-producing T cells. (**c**) Antibodies are circulating into the sera ready to be mobilized in case of infection. Memory B and T cells can recirculate and patrol through peripheral lymphoid organs and tissues. Effector CD4+ T cells release cytokines and interact with phagocytes. Antibody-coated pathogens are destroyed by the immune cells. **CTL** = cytotoxic T lymphocytes.

other innate cells, including monocytes and dendritic cells (DCs). These types of cells are known as APCs, which can take up antigen and, upon activation, migrate to the draining lymph node where they present the antigen to T cells. In addition, APCs can also be activated directly by some of these molecular patterns.[26] Altogether, APCs play a key role in integrating the information provided by the environment and in activating the T- and B-cell response in an appropriate manner (figure 1b). As a consequence, the modulation of innate immunity strongly influences the magnitude of T- and B-cell responses as well as their quality by influencing the type of cytokines made by effector T cells and the affinity of the antibody for the antigen.

Various adjuvant combinations – or AS – can be selected to target innate responses leading to adaptive immune responses appropriate to inducing protective immunity against specific pathogens in a selected target population. We report here the example of the development of the AS04-adjuvanted HPV-16/18 cervical cancer vaccine.

2. Designing a Vaccine Against Human Papillomaviruses (HPVs): Addressing the Challenge

Cervical cancer is the second most frequent cancer amongst women,[27] and infection with oncogenic strains of HPV is accepted as a necessary step for the development of cervical cancer.[28] Approximately 10–15% of HPV infections become persistent. Persistency is a key factor in the development of cervical neoplasia.[28-30] HPVs are classified by genotype, based on the DNA sequence encoding the L1 major capsid protein. There are at least 130 recognized variants,[31] with approximately 15 being classified as oncogenic.[32] The predominant oncogenic types are HPV-16 and -18, which account for approximately 70% of cervical cancer cases.[33] However, other HPV types have also been implicated in the development of cervical cancer, noticeably HPV-45, -31, and -33. These HPV types, which belong to the same phylogenetical high-risk group as HPV-16 and -18, represent the next most prevalent types besides HPV-16 and -18 worldwide, and cause an additional 10% of cervical cancer cases.[34]

2.1 The Challenges of HPV as a Pathogen

Cervical HPV infection remains localized within the mucosal epithelium. It is asymptomatic and the virus life cycle does not include viremia, thus limiting exposure of the antigen to the immune system. The HPV genital types have a highly specific tropism for the basal layer of the squamous epithelium of the mucosal surfaces (figure 2). Replication of the viral genome occurs in the lower epithelial layers,[35,36] and as the epithelial cell reaches the end of its life cycle, it spontaneously releases new virus particles into the lumen.[35] This avoids virus-induced cell lysis, thus preventing initiation of inflammatory responses.[35,36] The virus remains largely hidden from the immune system and the adaptive immune response is attenuated, although the majority of naturally acquired infections are cleared.[37]

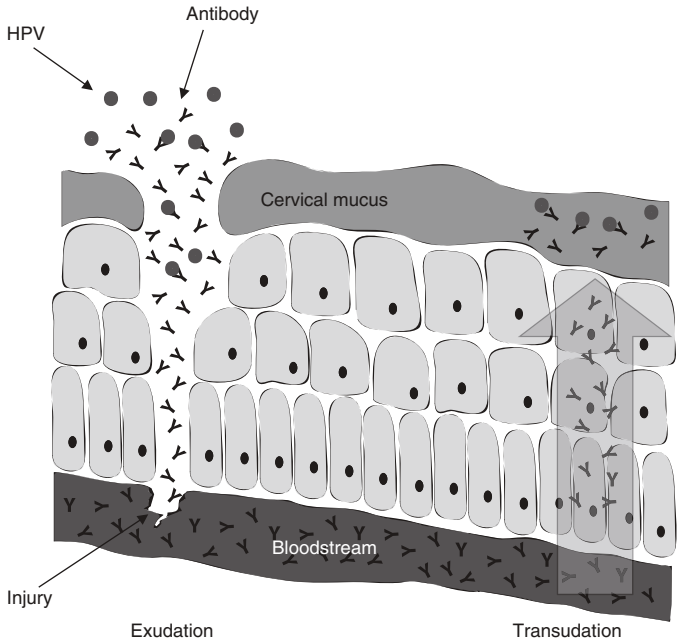

Fig. 2. How circulating antibodies can protect against human papillomavirus (HPV) infection. After immunization through the parenteral route (intramuscular injection) and the generation of a high circulating antibody response, vaccine-induced protection is mediated by antibodies that either exudate through microabrasions or transudate to the cervical mucosa.

Natural immune responses following infection do not always reliably protect against subsequent HPV infection or eliminate the risk of a HPV infection becoming persistent; hence, the risk of reinfection is lifelong.[38,39] Results of a recent study also suggest that natural immunity is not sufficient to control reinfections.[40] Virus-host interactions and life-cycle features of HPV, therefore, present a challenge to effective vaccination.

2.2 HPV Vaccine Target Populations

The risk of HPV infection is present from the beginning of sexual activity; hence, the optimum target population for HPV vaccination is HPV-naive young women who have not yet been exposed to the risk of infection. This is reflected in the organized vaccination programs and recommendations that

preferentially target adolescent girls. Young and adult women who may have been exposed to HPV can also benefit from HPV vaccination, as previous exposure does not reliably provide protection against reinfection with the same type or other virus types.[40] The characteristics of the target population are an important consideration in designing an effective vaccine against HPV. Indeed, because of the age of the target population, the vaccine will preferably induce a long-lasting immune response.

2.3 Development of an HPV Vaccine

The first challenge for the development of an HPV vaccine is to achieve protective efficacy through induction of a systemic immune response against a virus that enters and remains localized only in the mucosa.

The antigenic component of the AS04-adjuvanted HPV-16/18 cervical cancer vaccine consists of virus-like particles (VLPs) from the L1 capsid protein of the two most frequent oncogenic types, HPV-16 and -18, which are adsorbed on aluminum salts.[41] The L1 proteins self-assemble into VLPs, which renders them more immunogenic than linear recombinant L1 extracted from bacteria,[16,41] and closely mimic the HPV virions.[41,42] The recognition of L1 depends on the conformation of the proteins that is not preserved in denatured proteins or peptide fragments. Importantly, adsorption of the VLPs onto aluminum hydroxide does not alter their conformational structure.[43] The selection of L1 protein as vaccine antigen was based on the observation in animal studies that antibodies against the L1 component were able to neutralize authentic virions and that this protection could be passively transferred to HPV-naive animals.[44-46]

Animal challenge data with canine oral papillomavirus have shown that high levels of papillomavirus-specific neutralizing antibodies in serum also provides mucosal protection against infection,[46] as systemically induced antibodies access the cervical mucosa via transudation or exudation mechanisms.[47]

AS04 was selected as the AS for the HPV vaccine to secure enhanced priming of the immune system in order to afford high protection for as long as possible – potentially for an entire lifetime. The amount of HPV-16 and -18 antigens were optimized to avoid possible interference.

As adolescents are to be vaccinated prior to sexual debut, it is crucial that optimal protection be sustained once they are exposed to the virus and as long as they are exposed to the virus. Selection of AS04 for AS04-adjuvanted HPV-16/18 cervical cancer vaccine was supported by clinical experience with the first hepatitis B virus (HBV) vaccine formulated with AS04 registered in Europe.[48] This vaccine was developed for pre-hemodialysis and hemodialysis patients, who are particularly at risk of HBV infection and often require multiple doses of standard HBV vaccine in order to obtain adequate protection.[48,49] Studies in these populations have demonstrated higher seroprotection rates and longer-lasting antibody response to the HBV vaccine adjuvanted with AS04 than with the classical HBV vaccine formulated with aluminum salt.[50]

AS04 is composed of MPL adsorbed onto aluminum salts. MPL is a derivative of the lipopolysaccharide moiety of the Gram-negative bacterium *Salmonella Minnesota*,[51] and is known to be a highly specific agonist of the TLR4 receptor.[1,52,53] It is the first TLR agonist to be licensed as part of a vaccine for human use.[23,53]

3. AS04-Adjuvanted HPV Vaccine: Proof of Concept Studies

As the AS approach represented a new concept in HPV vaccine development, initial studies focused on demonstrating the enhanced benefits of HPV vaccines formulated with AS04 compared with classical formulations. When comparing the same HPV VLPs formulated either with aluminum hydroxide or with AS04 in mice, monkeys, and humans, the formulation with AS04 demonstrated enhanced and sustained priming of both humoral and cellular immune responses, including higher antibody levels in humans up to 3.5 years after vaccination and a higher frequency of memory B cells in all species.[16] In contrast to published reports on the ability of HPV VLPs to trigger TLR4,[54] HPV-16 and -18 VLPs in the AS04-adjuvanted vaccine did not activate APCs *in vivo* nor *in vitro*.

4. AS04 Targets the Innate Immune System and Hence Influences the Quality and Magnitude of the Adaptive Immune Response

Extensive experimental work has been carried out in mice *in vivo* and on human cells *in vitro* to characterize the mechanism of action of AS04 in the context of the AS04-adjuvanted HPV-16/18 cervical cancer vaccine[1] (see figures 3 and 4 for a comparison of mechanisms of action of vaccines adjuvanted with aluminum and AS04). It was observed in mice that the optimal immune response to the vaccine was achieved when both components of the vaccine, i.e. AS04 and the L1 VLP antigen, were spatially and temporally co-localized.[1] The capacity of AS04 to enhance HPV antigen-specific humoral response was optimal during the first hour after injection and totally disappeared 24 hours after injection. Moreover, it was demonstrated that

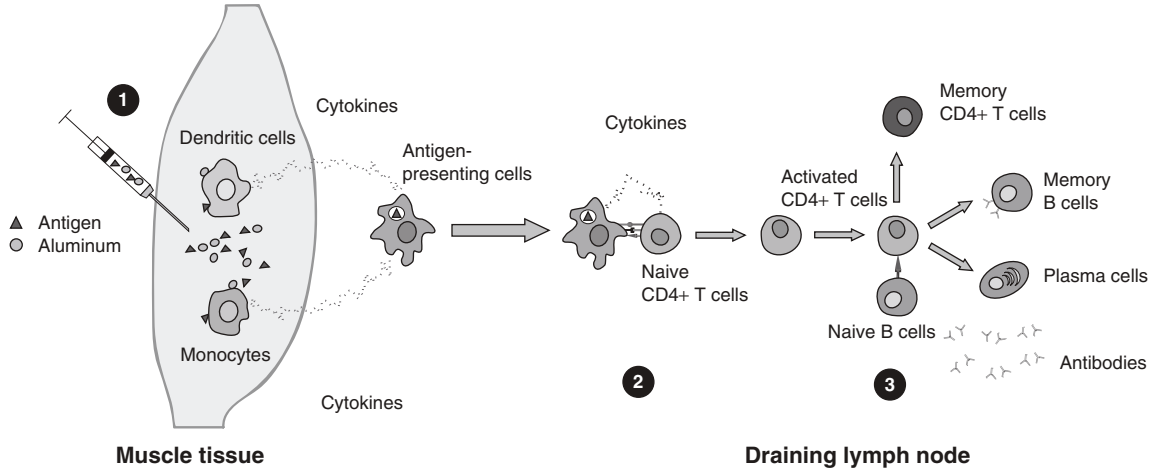

Fig. 3. Mechanism of action of aluminum adjuvant. Step (**1**): The vaccine is injected into the muscle. Aluminum impacts on activation of monocytes and possibly dendritic cells, and also on antigen uptake. Step (**2**): Activated monocytes and/or dendritic cells migrate to the draining lymph node where they present the antigen to CD4+ T cells. Antigen-presenting cells also provide co-stimulatory and cytokine signals to the T cells in order to promote the stimulation of T cells. Step (**3**): Activated CD4+ T cells support the induction of B-cell responses.

AS04 had no capacity to enhance antibody response against HPV antigens when injected at a different immunization site.[1] The impact of AS04 on vaccine antigen-specific response was thus local and transient. In the clinical situation, both components of the vaccine are administered in a single injection, thereby ensuring co-localization.[1]

In mice, intramuscular injection of MPL or AS04 led to the local production of cytokines and chemokines detected in muscle and draining lymph nodes but not in the serum of the mice. In line with these results, the MPL and AS04 were shown to induce the activation of the transcription factor nuclear factor κB (NF-κB), which is a master switch of the inflammation.[55] Indeed, NF-κB is known to control a range of cellular processes, including the production of cytokines and chemokines required for the initiation of an innate immune response. As shown by imaging technology, the induction of NF-κB by AS04 was limited to the injection site and the draining lymph node.[1]

The principal component of AS04 responsible for the early activation of APCs and production of cytokines/chemokines was shown to be MPL dependent. Although both MPL and aluminum salts were required to get the optimal humoral response to the VLPs, aluminum salt did not seem to contribute quantitatively or qualitatively to the peak of the local innate response but rather prolonged the innate response of MPL.[1] The higher local production of cytokines induced by AS04 correlated with the increased number of activated DCs and monocytes carrying the antigen in the draining lymph node, as compared with aluminum salts. In addition to its impact on DC recruitment and activation, AS04 increased the ability of DCs to stimulate antigen-specific T cells, compared with aluminum salts. All these data can explain the higher immunogenicity of the AS04-adjuvanted vaccine compared with the aluminum salts-based vaccine.

It has also been demonstrated that AS04 does not act directly on T cells to activate or co-stimulate them.[1] Although a recent study describes the expression and functionality of TLR4 on B cells in subjects with chronic inflammation,[56] TLR4 is not expressed on naive or memory B cells in healthy subjects, and therefore TLR4 agonists are unable to directly activate B cells in these individuals.[57-59] Together with the transient and localized aspect of the innate response induced by AS04, all these elements contribute to support the clinically acceptable safety profile of the HPV vaccine.

5. Clinical Profile of AS04-Adjuvanted HPV-16/18 Cervical Cancer Vaccine

The observed clinical profile confirms that the AS04-adjuvanted HPV-16/18 cervical cancer vaccine has favorably addressed the challenges associated with effective protection against HPV.[60-63] Currently available data show the vaccine to be highly immunogenic and efficacious against infection and lesions associated with HPV-16 and -18, with long-term data available up to 7.3 years following vaccination of HPV-naive women aged 15–25 years. The longer term extent of the persistence of immune response against oncogenic HPV-16 and -18 is still being evaluated in several cohorts.

The vaccine also induced cross protection against infection and/or lesions associated with HPV types that are phyloge-

netically related to HPV-16 (i.e. HPV-31 and -33) and HPV-18 (i.e. HPV-45). Additional analyses of vaccine efficacy against cervical intraepithelial neoplasia 2+ associated with these oncogenic types showed that cross-protective efficacy of the vaccine may represent an additional 11–16% of protection against cervical cancer.[64] However, the exact role played by the vaccine formulation on the magnitude of the observed cross-protective effect remains to be determined.

As organized vaccination programs preferentially target young girls prior to sexual debut, it is also of importance to bridge efficacy results observed in young women aged 15–25 years. Immunologic bridging studies have been accepted to infer efficacy in younger girls. This was done in a study evaluating the immune response in young girls aged 10–14 years versus 15–25 years, where the vaccine was shown to induce serum antibody levels in younger girls that were twice as high as those in the 15- to 25-year-old population.[65]

Because of the lifelong risk of re-exposure to HPV and exposure to multiple strains, 'catch-up' programs may be used in order to offer the benefits of HPV vaccination to older women, including those who may have been previously exposed to HPV. The HPV-16/18 vaccine was shown to induce 100% seroconversion for both antigens when administered to women aged 15–55 years. In the same study, a high correlation was observed among antibody levels in serum and antibody levels in cervicovaginal secretions.[66] In addition, the immunologic added value of AS04 as opposed to aluminum salt alone has been observed both in the first comparison of the aluminum-based and AS04-adjuvanted HPV vaccines, and then by a head-to-head comparison of the AS04-adjuvanted HPV-16/18 cervical cancer vaccine with an HPV L1 VLP vaccine formulated with an amorphous aluminum hydroxyphosphate sulfate (AAHS) as adjuvant.[67] This study in women aged 18–45 years demonstrated that the immune response tested after the full course of vaccination with the AS04-adjuvanted HPV-16/18 cervical cancer vaccine was superior ($p < 0.0001$) to the AAHS-adjuvanted HPV L1 VLP vaccine with respect to HPV-16 and -18 antibody levels as measured by pseudovirion-based neutralization assay (table I).

In addition, levels of antigen-specific memory B cells (a marker of persistence of humoral immune response) were 2.7-fold higher for the AS04-adjuvanted HPV-16/18 cervical cancer vaccine than for the AAHS-adjuvanted HPV L1 VLP vaccine group for both HPV-16 and -18 ($p < 0.0001$). The clinical importance of these differences in immune responses and whether the adjuvant has an impact on the magnitude of the observed effect is currently unknown.

6. Safety Profile of AS04 and the AS04-Adjuvanted HPV-16/18 Vaccine in Clinical Studies

Investigation into the mode of action of AS04 has provided supportive evidence for an acceptable safety profile of AS04.[1] AS04 acts locally and targets the activation of innate immune

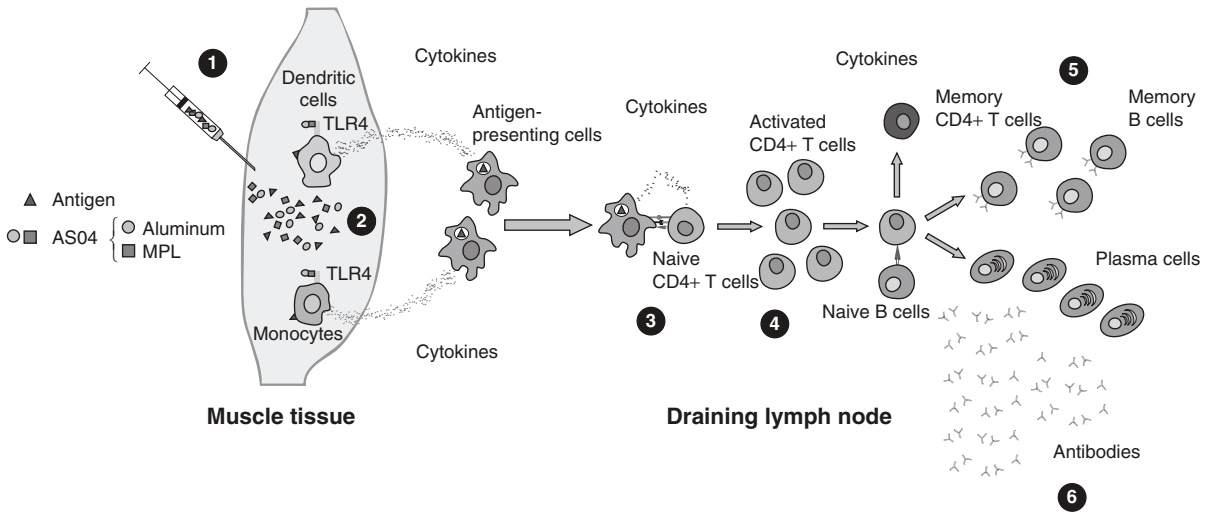

Fig. 4. Mechanism of action of AS04 adjuvant. Step (**1**): The vaccine is injected into the muscle. AS04 contains aluminum salts and monophosphoryl lipid A (MPL), a toll-like receptor 4 (TLR4) agonist. TLR4-expressing cells in the muscle, such as resident or recruited dendritic cell or monocytes, are activated and induce a local and transient response. This response is mainly driven by MPL. AS04 allows for the rapid recruitment and activation of monocytes and dendritic cells. Step (**2**): AS04 and virus-like particles co-localize. Step (**3**): Activated monocytes and dendritic cells, loaded with the antigen, migrate to the draining lymph node. AS04 allows for a better activation of those antigen-presenting cells, e.g. an enhanced expression of co-stimulatory molecules, which translates into an enhanced ability to present antigen to CD4+ T cells. Step (**4**): The generation of more and/or enhanced CD4+ T cells leads to a better differentiation of B cells. Step (**5**): AS04 increases frequency of antigen-specific memory B cell. Step (**6**): High levels of antibodies are released in the circulation.

Table I. Geometric mean antibody titers (GMTs) for human papillomavirus (HPV)-16 and -18 serum neutralizing antibodies

Patient age group (y)	GMT AS04-adjuvanted HPV vaccine	GMT AAHS-adjuvanted HPV vaccine	GMT ratio (97.6% CI)
HPV-16			
18–26	31 715	8 682	3.7 (2.7, 5.0)
27–35	25 134	7 322	3.4 (2.4, 5.0)
36–45	21 874	9 828	2.2 (1.6, 3.1)
HPV-18			
18–26	13 732	1 886	7.3 (5.2, 10.2)
27–35	9 390	1 178	8.0 (5.5, 11.6)
36–45	9 760	1 709	5.7 (4.0, 8.1)

AAHS = amorphous aluminum hydroxyphosphate sulfate.

cells at the site of injection, and its effect is limited in time. Additionally, there is no evidence of activation of interferon-α, a cytokine that has been associated with autoimmune diseases.

In an integrated analysis of data from 11 trials involving approximately 30 000 women (16 142 of whom received at least one dose of the AS04-adjuvanted HPV-16/18 cervical cancer vaccine), solicited symptoms and adverse events were analyzed.[68] As expected, there was a higher rate of solicited symptoms, mainly at injection site, in the AS04-adjuvanted HPV-16/18 cervical cancer vaccine group than in control groups [$Al(OH)_3$ or hepatitis A vaccine], and this may be explained in part by the impact on the innate immune response at the local level.

Because of the addition of adjuvants, attention is increasingly being paid to the theoretic association with autoimmune diseases. This is in part due to the temporal association of vaccination and the onset of the autoimmune diseases, which may be incorrectly interpreted as a causal relationship.[69] As autoimmune diseases are rare events that require a larger sample size for their evaluation, a large integrated safety analysis was performed to evaluate autoimmune diseases in the context of vaccination with ASO4-adjuvanted vaccines. The analysis included 68 512 individuals from all randomized, controlled trials of registered GlaxoSmithKline Biologicals products and candidate vaccines containing AS04.[70] Overall reporting rates of autoimmune diseases were approximately 0.5% and did not differ significantly between AS04 and control groups [non-adjuvanted vaccines, $Al(OH)_3$-adjuvanted vaccines, or $Al(OH)_3$]. The relative risk (RR) of experiencing any autoimmune disease was 0.98, and RRs calculated overall for disease category or for an individual event were close to 1, meaning that there is no increased risk associated with AS04-adjuvanted vaccines. An additional analysis was performed in the same study on data for 39 160 individuals with the AS04-adjuvanted HPV-16/18 cervical cancer vaccine alone. These data also showed that there was no indication that AS04-adjuvanted HPV vaccine may increase the risk of autoimmune disease (RR = 0.92; 95% CI 0.70, 1.22).[70]

Overall, these data indicate that there is no evidence of a causal association between autoimmune diseases and AS04, and that the AS04-adjuvanted HPV-16/18 cervical cancer vaccine has an acceptable safety profile in women of all ages. Postmarketing surveillance is in place to further monitor, in the long-term, the safety profile of the AS04-adjuvanted HPV-16/18 vaccine.

7. Conclusions

Identifying the best vaccine strategy for each pathogen and specific population is challenging. Recent progress in adjuvant and antigen design has offered powerful tools to address these challenges. One recent approach is the formulation of purified antigens with AS, combining more than one adjuvant. The formulation of antigens with the appropriate AS triggers innate immune signals that translate into an enhanced specific adaptive immune response.

The AS04-adjuvanted HPV-16 and -18 cervical cancer vaccine induces an enhanced and sustained immune response against both vaccine antigens in women aged 15–55 years. High levels of neutralizing antibodies are induced and detected at the cervical mucosa due to a passive transfer from the serum. The vaccine shows high and sustained protection against infections and precancerous lesions caused by HPV-16 and -18 and cross protection against some other oncogenic types not contained in the vaccine, along with an acceptable safety profile. The mechanism of action of AS04 in the AS04-adjuvanted HPV-16/18 cervical cancer vaccine provides a molecular- and cellular-based rationale for the observed immunogenicity, efficacy, and safety of this formulation.

The process of selecting the appropriate combination of antigens and adjuvants by considering the specificities of both

the pathogen and the host holds significant promise in the fight against diseases and represents a next step in the evolution of vaccine science.

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