Designing Vaccines against Human Papillomavirus and Hepatitis B Virus: Similarities and Differences for Preventable Viral Infections and role of AS04 Adjuvant System in Addressing Specific Challenges

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Abstract

Pathogen characteristics and the mechanism of host/pathogen interaction play a key role in design, formulation, and development of vaccines. Here we present the experience of GSK Bio in developing two vaccines with a novel Adjuvant System (AS04) against two viral infections, Human papillomavirus (HPV) and Hepatitis B virus (HBV), that have some similarities and many differences.

Developing a vaccine against HPV is difficult because the virus remains local, evades the immune system, and does not induce a reliable long lasting protection upon natural infection. Vaccination of pre-haemodialysis and haemodialysis patients against hepatitis B represents a challenge as well, because these patients are immunocompromised and develop a reduced and short lasting immune response to administration of conventional HBV vaccines.

This opinion paper describes how a novel adjuvant, AS04, has been introduced in the formulation of HPV and HBV vaccines in order to address the challenges represented by the virus (i.e., HPV) or the population (i.e., HBV) targeted by vaccination. Clinical results from these vaccines will be described in light of other vaccines containing more conventional adjuvants, such as aluminium salts.

Adjuvants can be used to amplify the immune response to vaccine antigens. The combination of antigens with more than one adjuvant (referred to as "Adjuvant System"), can lead to the development of vaccines which generate specific and effective immune responses adapted to both the pathogen and the target population. Among those Adjuvant Systems, AS04, a combination of the TLR4 agonist MPL and aluminum salt, is contained in two licensed vaccines against HPV and HBV.

The use of HBV and HPV vaccines formulated with AS04 has been shown to enhance the immune responses in vaccines, while maintaining a clinically acceptable reactogenicity and safety profile.

Keywords: HPV; HBV; Vaccines; AS04; Adjuvants; Adjuvant system

Introduction

Several intracellular pathogens can establish long-lasting chronic infections and may lead to clinical disease. Two examples of such pathogens are the Human papillomavirus (HPV) and the Hepatitis B virus (HBV). Immunological mechanisms are involved both in the establishment and maintenance of the tolerance to these infections. Chronic infections and diseases are the results of a delicate interplay between the pathogen’s actions to survive in the host and the attempts of the immune system to eradicate it.

HPV is one of the most common chronic viral infections in humans. The virus consists of circular, double stranded DNA and has no envelope. Approximately 130 different HPV types have been identified so far [1] and about 40 of these infect the human genital tract, including 15 of types considered as oncogenic [2]. It is estimated that up to 80% of women will acquire a HPV infection in their lifetime [3]. Cervical HPV infections are asymptomatic; although most of them clear spontaneously and only 5–10% will become persistent infection possibly leading to the development of clinical pre-cancerous lesions and cancer.

Upon natural infection, HPV remains at the site of infection, evades the immune system, and does not reliably induce protective immunity as the virus does not kill infected cells and hence neither inflammation nor release of danger signals to be recognised by the immune system is triggered. As a result, new infections and re-infection can occur [4]. A prophylactic HPV vaccine should therefore induce a better immune response than natural infection by overcoming the challenges due to the pathogen (i.e. have the ability to block the virus at the site of entrance), hence induce higher level of antibodies, and provide long-term protection [4].

HBV infects hepatocytes and consists of a double-stranded circular DNA genome, an outer envelope protein (HBsAg), an inner nucleocapsid protein, (HBcAg), and a soluble small molecular weight protein (HBeAg) produced by the core gene. The HBsAg envelope protein can be shed and is also found as a non-infectious self-assembling tubular or spherical particle in the bloodstream of infected patients [5]. Overall eight genotypes of HBV are known [6,7].

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In addition to challenges due to pathogen features, the challenges of HBV vaccination are also due to the host characteristics, i.e. poor immune response of pre-hepamodialysis and haemodialysis patients to standard HBV vaccination as a consequence of their impaired immune system. This is of particular concern since these patients are at increased risk of HBV infection during dialysis procedures [8]. In this population, double dosage of vaccine in a 4 dose schedule is recommended, followed by regular booster when the antibody level declines below the protective level. This contrasts with the recommendations in healthy subjects, consisting of 3 or 4 doses with no boosters [9].

The need for improved vaccine formulations for challenging immunological requirements and populations led GSK Bio to develop HPV and HBV vaccines adjuvanted with the AS04 Adjuvant System. The choice of AS04 was based on the results from preclinical and clinical studies, which consistently showed a higher antibody response with the AS04-adjuvanted formulations as compared to aluminium-containing formulations [10-12].

### Similarities and Differences between HPV and HBV Infections

#### HPV infection

HPV is mainly transmitted by sexual contact, and remains located in the epithelial cells of the mucosa or the skin. There is little, if any, exposure of the virus to the host’s immune system because HPV proteins are expressed at low levels and not secreted [13-15]. The natural infectious cycle of HPV is adapted to the differentiation program of the infected cell. Basal keratinocytes mature vertically through the epithelium to the cervical lumen. The time from infection to viral release is approximately 3 weeks, which coincides with the time for basal keratinocytes to undergo complete differentiation, desquamation and natural cell death. The adaptation of HPV to the differentiation program of keratinocytes is an important mechanism as it allows the virus to evade detection by the immune system. The virus by itself is not cytolytic, therefore natural cell death does not present a danger signal to the immune system nor is it accompanied by inflammation [2]. During maturation, the late proteins L1 and L2 are expressed to form capsids and viruses are only shed externally [16].

As a result of these and other host evasion mechanisms, the local innate immune responses aiming to control or eradicate the virus are attenuated, allowing the infection to become persistent [2,17]. Some of the viral antigens have cell-transforming properties that can lead to the development of benign or malignant tumours, if the infection persists [18]. HPV viral load measurement may also be predictive of future cervical neoplasias (CIN1), to high grade cervical intraepithelial neoplasias (CIN2 and CIN3), e.g. cancer in situ (CIS) and ultimately to invasive cervical carcinoma (CC). Only a small percentage will progress from CIN2 to CC and this process usually takes several years. At each stage, with the exception of CIS and CC, the infection may revert to the previous stage, although therapy is recommended from stage 2 onwards. In most cases total clearance of lesions and infection has been observed, but the rate of spontaneous clearance decreases with the severity of the precancerous lesions [46].

#### HBV infection

HBV is a systemic pathogen with a specific tropism for the hepatocytes but it can also be found in the blood and in other cells and tissues [20]. The infection with HBV is a complex process and is still not fully understood [21]. A large amount of virus can be produced by infected liver cells without causing a cytopathic effect, even though up to 10^11 virus particles may circulate in the bloodstream during infection. The infected cells become a target for the immune system causing liver cell damage and resulting clinical symptoms of hepatitis disease [20].

Acute infection may evolve into chronic infection if T cell responses to HBV are not induced or not capable of controlling the virus [22]. T cell responses to HBV antigens disappear gradually with the onset of chronic infection, probably because of exhaustion and/or tolerance induction [23]. The transition from acute to chronic infection appears to represent a failure of immune clearance of virus-infected cells and is marked by persistence of high levels of HBV DNA and HBsAg in serum [20]. HBV infection can therefore either lead to acute hepatitis disease followed by clearance of the virus, or to the persistence of the virus and chronic, but often subclinical, infection. The latter type of infection can be maintained for many years and may lead in the long term to complications such as liver fibrosis or hepatocellular carcinoma (HCC). The inactive carrier state generally has a benign course. However, the infection can be reactivated either spontaneously or by immune suppression [24-26]. The re-activation of the infection can be abrupt and resemble acute hepatitis [27]. CHB is a lifelong infection, which evolves over time in response to changes in the balance between the immune response and the viral activity.

### Progression of Chronic Infections with HBV and HPV to Malignant Cancers

Infections with HPV or HBV may result in the development of malignant tumours with a significant mortality rate.

#### HPV

Cervical cancer is the second most common cancer in women worldwide [3]. About 500,000 new cases of cervical carcinoma occur every year worldwide [28,29]. All cervical cancers are associated with HPV infection [29,30] and persistent infection with an oncogenic type of HPV is an important early precursor event in the progression to cervical precancerous lesions and cervical cancer [18,31-36]. A persistent infection is generally defined as the continued detection of viral DNA of the same HPV type in the cervix for at least 6–12 months [37-39].

HPV types are classified into low risk HPV, i.e., which do not lead to neoplastic transformation and high risk or oncogenic HPV, i.e., which are associated with the development of cancers. Among the 15 oncogenic HPV types [34,40-43], HPV types 16 and 18 cause approximately 70% of all cervical cancer cases worldwide [18,40], followed by 45, 31 and 33 which cause an additional 10% of cervical cancer cases [44]. HPV can also cause other non-cervical carcinomas such as head and neck cancer [45].

Infection by oncogenic HPV can evolve to low grade cervical intraepithelial lesions (CIN1), to high grade cervical intraepithelial neoplasias (CIN2 and CIN3), e.g. cancer in situ (CIS) and ultimately to invasive cervical carcinoma (CC). Only a small percentage will progress from CIN2 to CC and this process usually takes several years. At each stage, with the exception of CIS and CC, the infection may revert to the previous stage, although therapy is recommended from stage 2 onwards. In most cases total clearance of lesions and infection has been observed, but the rate of spontaneous clearance decreases with the severity of the precancerous lesions [46].

#### HBV

Worldwide, more than 350 million individuals are chronically infected with HBV and up to 40% of infected patients will develop serious complications [47-49].

About 600,000 deaths each year are due to HBV-related acute hepatitis, liver fibrosis or HCC [50]. CHB is the first cause of liver cancer worldwide and the primary cause for cancer in men in Asia
HBV is the leading cause for HCC and core promoter mutations have been shown in many studies to be associated with increased risk of HCC and to precede HCC diagnosis [52].

It is well documented that the chance of an acute versus chronic infection is dependent on the age at infection. Young children who become infected with hepatitis B virus are the most likely to develop chronic infection. About 90% of infants infected during the first year of life and 30% to 50% of children infected between 1 and 4 years of age will develop chronic infection. The situation is different in adults. At the onset of HBV infection, 35% of adults show symptoms of acute hepatitis whereas the other 65% have sub-clinical disease [25]. About 5% of adults develop CHB, but the rates of chronicity are much higher in immune deficient individuals. About 60% of patients on haemodialysis develop CHB when infected with HBV compared to 5-10% of healthy adults [53,54].

**Differences in Immune Responses after Natural Infection for HPV and HBV**

**HPV**

As HPV infections are usually limited to their host cells, the keratinocytes, the virus is barely exposed to the systemic immune system due to the absence of a bloodstream phase and the host is unable to mount a strong antibody response. Innate cell-mediated immunity is the primary mechanism of defence for HPV infection, adaptive response comes into play at a later stage. Antibody levels induced by natural infection are very low, but the antibody levels increase in individuals with persistent infections [55]. Antibody levels observed after natural infection seem to be unreliable for long-term protection. Hence, it has been observed that seropositive individuals can be reinfected with the same type of HPV [56,57]. However, recent data from natural history evaluations show that the reduced risk of a new infection can be associated with high levels of antibodies [58]. The limited innate immune response, the low level of viral gene expression in the basal epithelium, and the lack of cytopathic effects result in a delayed adaptive immune response.

The degree of protection and the duration of immunity induced by natural incident infection are not known, but only 50–60% of women develop detectable serum antibodies to HPV after natural infection [17,59]. A cell-mediated immune response of effector T lymphocytes directed against the early E2 and E6 HPV proteins occurs first, as reflected by the infiltration of specific helper and cytotoxic T lymphocytes, macrophages, and the local production of proinflammatory cytokines [4,60,61]. Approximately 8 months after infection, low levels of neutralizing antibodies to the major capsid protein L1 may appear in the serum of infected individuals, and specific IgG and secretory IgA are found locally in the cervical mucus, but at very low levels [62-64].

**HBV**

HBV immune response is distinct from what is observed with the HBV infection, where a rapid anamnestic response can occur upon virus challenge, even in the absence of detectable specific antibody titres at the time of infection [65]. One important difference between HPV and HBV viral infections is that HBV shows persistent viraemia during the course of the infection. The rapid presence of viral particles in the bloodstream allows the immune system to recognize the pathogen and to mount a rapid and strong response against the pathogen.

The immune response to HBV infection includes cellular response and the secretion of high levels of circulating antibodies to neutralize and control the pathogen [20,66,67]. During acute hepatitis B disease, a strong cytotoxic T lymphocyte (CTL) response is detected [68]. This CTL response is directed against multiple epitopes within the envelope, polymerase, HBc and HBe antigens [66,69-73] and leads to the destruction of hepatocytes.

During the early phase, Kupffer cells, NK cells, NK T cells are activated in the liver and contribute to the early clearance of the virus through secretion of type 1 interferons [74].

Cell-mediated immunity is crucial for the control of HBV infection [20]. Strong virus-specific CD4+ and CD8+ T cell responses are induced during transient infection and these responses can persist for decades after viral clearance [75]. Patients who spontaneously recover from HBV infection typically mount vigorous specific CD4+ and CD8+ T cell responses whereas those with CHB have late, transient or narrowly focused T cell responses [66]. The continued presence of T cells suggest that HBV persists at low levels despite absence of detectable HBV DNA and presence of antibodies to HBsAg in serum [76]. Reactivation of hepatitis B during immunosuppressive therapy in people who had previously cleared an HBV infection has been amply documented [77,78].

The main similarities and differences for HPV and HBV infections are summarised in Table 1.

**The Challenges of HPV and HBV Vaccinations**

**HPV**

The mechanism of protection against HPV infection is not completely understood, but it has been shown in animal models that high levels of HPV-specific neutralising serum antibodies play an important role in preventing infection [79-81]. This provided the necessary evidence that a vaccine inducing neutralising antibodies could be developed for humans. These antibodies are not produced locally, but result from the transudation or exudation from serum to the cervical mucus. The levels of antibodies in the cervix have been shown to correlate with the levels of antibodies in the serum, supporting passive transfer of antibodies in the cervix (Figure 1) [82-84].

<table>
<thead>
<tr>
<th>Similarities</th>
<th>Chronic infection can lead to cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Differences</td>
<td>HPV</td>
</tr>
<tr>
<td>Local infection at the mucosa</td>
<td>Systemic infection</td>
</tr>
<tr>
<td>Attenuation of immune mechanism</td>
<td>Viral progression 1 to 6 months from infection to disease</td>
</tr>
<tr>
<td>Many viral subtypes</td>
<td>Strong immune response</td>
</tr>
<tr>
<td>Viral entry occurs rapidly, but infected cell shows no inflammation</td>
<td>Neutralising antibodies and CTL activation</td>
</tr>
<tr>
<td>Virus elimination via natural cell clearance</td>
<td>Immune mechanisms in subjects who recovered from HBV infection provide lifelong immunity</td>
</tr>
<tr>
<td>Innate immune response, late induction of adaptive response (low antibody levels after long exposure to infection)</td>
<td></td>
</tr>
<tr>
<td>Natural immunity does not reliably prevent from infection</td>
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</table>

Table 1: Similarities and differences HBV and HPV infection.
In order to provide long-term protection against HPV infection and associated lesions, prophylactic HPV vaccines should elicit an appropriate immune response in terms of antibodies and memory B cells. The first challenge of HPV vaccination is to provide protective efficacy through a systemic immune response against a virus that enters only via the mucosal route and remains localized. The antibodies must not only reach the cervical mucosa, but they also need to be sustained at sufficient levels and in a timely and spatially orchestrated manner to neutralize the virus before it enters the cells. In that respect, antibody titres inferior or even close to those induced by natural HPV infection are unlikely to be sufficient. Currently, two prophylactic HPV vaccines are available (Table 2).

### HBV

Efficacious HBV vaccines are available for more than 20 years (Table 2). About 95% of healthy young individuals respond to vaccination with the standard hepatitis B vaccines. However, HBV infection is still a major health problem worldwide [85]. Compared to adults with normal immune status, pre-haemodialysis and haemodialysis patients have a suboptimal response to standard HBV vaccination with aluminium adjuvanted vaccines, because their immune system is impaired. These patients require double dosage of HBV vaccine and a four-dose schedule, followed by frequent boosters to maintain protection [86-90]. For that reason GSK Bio developed an alternative HBV vaccine formulation targeted to this specific population [91]. Up to 60% of infected patients on haemodialysis are not able to clear the virus and therefore become chronic HBV carriers [92]. Moreover, even though patients are vaccinated and appropriate measures to prevent transmission of the virus have been put in place, HBV outbreaks in dialysis centres still occur [93,94].

In the normal healthy population, long-term protection relies on immune memory in humans reaching anti-HBs concentrations ≥10 mIU/mL post vaccination.

For immunocompromised individuals, protection against HBV infection relies more on circulating antibodies [95]. Studies in haemodialysis patients showed that many patients with anti-HBs levels between 10 and 100 mIU/mL did not retain protective antibody level of ≥10 mIU/mL one year post vaccination. It has therefore been suggested that anti-HBs concentrations ≥100 mIU/mL represented a more reliable indicator after vaccination, allowing protection to last for one year post vaccination [95,96].

The main similarities and differences for HPV and HBV vaccine formulations as described above are summarised in Table 3.

### The Role of B-Cell Memory in Long Term Protection

Naïve B lymphocytes differentiate into antigen-specific memory B cells and plasma cells after stimulation by the antigen through the B cell receptor followed by cognate T cell help. Memory B cells survive in secondary lymphoid organs in the absence of antigen and mediate secondary immune responses upon re-encounter with the antigen. Plasma cells are terminally differentiated cells that home to spleen and bone marrow and secrete high rates of antibodies. Traditionally, the induction of memory B cells is considered as a crucial factor for long-term vaccine-induced protection [4]. Recent studies have demonstrated a positive correlation between the frequency of antigen-specific B-cell memory and antigen-specific serum antibody levels in studies of vaccines against tetanus toxoid [97], smallpox [98] and hepatitis B [99].

The biology of plasma cells, which secrete these antibodies, is still not completely understood and under considerable debate [100]. Of particular interest for this discussion is the question about the respective role of memory B cells and long lived plasma cells in persistence of humoral response [97,101-103]. Three different concepts to explain persistence of humoral response have been postulated:

- short-lived plasma cells are generated continuously from memory B cells, a process that would be driven by persisting antigen [103]
- long-lived plasma cells with a defined half-life are generated from memory B cells, a process that would be activated by signals from cytokine receptors and Toll-like receptors by a new encounter with the pathogen [97,98]
- plasma cells can stay for a long time in special survival niches in the bone marrow [100,102]. This mechanism is currently considered as the most important since depletion of memory B cell has been shown not to significantly impact level of circulating antibodies [104,105].

The generation of memory B-cells and their response to antigen recall are crucial factors for the long-term efficacy of vaccine-induced humoral protection. B-cell memory and its generation are poorly understood. The rapid clonal expansion of B-cells in the lymph node follicle leads to the formation of the germinal centre where the key event of affinity maturation for the generation of high-affinity B-cell receptors (BCR), and thus high-affinity antibodies, occurs. This event is critical for vaccines whose efficacy is dependent upon the generation of high-affinity neutralising serum IgG. At some point the maturing B-cells exit from the germinal centre and enter the long-term memory B-cell compartment. Upon antigen rechallenge, these memory cells rapidly expand and differentiate into plasma cells.

Testing for anti-HBs antibodies is often negative many years after vaccination, but protection is solid and long lasting though, as demonstrated in a follow-up study 20 years after vaccination of high risk infants in Thailand [106,107]. For HBV it has been demonstrated that postvaccination titres ≥10 mIU correlate with the induction of T-helper cell responses which mediate generation of B-cell memory. Natural exposure to HBV after vaccination therefore results in an anamnestic response that prevents disease symptoms from occurring and in many cases even infection [108]. Based on this observation...
Long persistence of protection needed to prevent new infections

Amorphous aluminium hydroxyphosphate sulfate

GSK Biologicals

Vaccination prevents from disease but not necessarily from infection

No serological correlate of protection identified

Role of B-memory and vaccine anamnestic response

HBV

Need to prevent infection

Need of local protection with high antibody level at site of infection

Neutralising antibodies at the site and time of exposure are likely to prevent viraemia and resides within the cervical epithelium, an anatomic barrier to oncogenic HPV through the cervix or genital tract is capable of triggering antibody production from memory B cells to prevent infections with pathological consequences. Since HPV infection does not induce viremia and resides within the cervical epithelium, an anamnestic response might not be a reliable mechanism of protection. Neutralising antibodies at the site and time of exposure are likely to provide the best protection against infection.

Because women are at risk for acquisition of HPV infection for as long as they are sexually active, vaccination needs to induce long-term protective efficacy. Serum neutralizing antibodies, which are known to transudate/exudate to the site of infection, are generally presumed to constitute the major basis of protection against HPV infection for prophylactic vaccines.

AS04 Adjuvant System in Vaccines against HBV and HPV

The careful selection of antigens and the addition of adjuvants to amplify the immune response is one of the most used approaches in vaccine design today. Many of recently developed vaccines contain purified antigens, obtained for example by recombinant technologies that have the advantages of a reduced reactogenicity as compared to the whole pathogen. However, the use of purified antigens do not always lead to the induction of an adequate immune response, as the purification process may have removed immunogenic components that are important in triggering the immune defence mechanisms [109]. The addition of adjuvants in that context proves useful in ensuring a good immune response along with an acceptable reactogenicity and safety profile.

Adjuvants are substances with immunostimulatory properties [110-113]. Aluminium salts were the first to be registered for use in humans and are still the most widely used adjuvants in vaccines in all age populations [114,115]. Their mode of action has been shown to rely on several mechanisms such as local inflammation and an improved uptake of the antigen by the antigen presenting cells (APCs). Aluminium salts can induce a sufficient antibody response but they are poor activators of dendritic cells (one of the main tissue resident innate cells that can become antigen presenting cells) and they induce weak cellular response with a preferential Th2 pattern [116,117]. Recently, aluminium salts have been found to activate components of the inflammasome complex [118-120] by inducing uric acid [121], but the role of the inflammasome in mediating the adjuvant properties of aluminium salts is still a matter of debate [120].

Some adjuvants interact with the Toll-like receptors family (TLR), represented by transmembrane signalling proteins expressed on many cell types, in particular on immune cells such as dendritic cells, allowing the immune system to detect infection [122,123]. A TLR

Table 3: Similarities and differences for HBV and HPV vaccines.

<table>
<thead>
<tr>
<th>Similarities</th>
<th>HBV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acceptable safety profile</td>
<td>Role of B cell memory established, protection observed in absence of circulating antibodies</td>
</tr>
<tr>
<td>Need for enhanced protection</td>
<td>Immunocompromised patients are in need of higher doses and of booster dose</td>
</tr>
<tr>
<td>Long persistence of protection needed to prevent new infections</td>
<td></td>
</tr>
<tr>
<td>Strong immune priming needed</td>
<td></td>
</tr>
<tr>
<td>Vaccination prevents from disease but not necessarily from infection</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Examples of HBV and HPV vaccines.

<table>
<thead>
<tr>
<th>Product</th>
<th>Manufacturer</th>
<th>Antigen</th>
<th>Adjuvant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gardasil®</td>
<td>Merck &amp; Co</td>
<td>Human papillomavirus (types 6, 11, 16, 18) recombinant vaccine</td>
<td>Amorphous aluminium hydroxyphosphate sulfate [161]</td>
</tr>
<tr>
<td>Cervarix™</td>
<td>GSK Biologicals</td>
<td>Human papillomavirus (types 16, 18) recombinant vaccine</td>
<td>AS04 (3-O-desacyl-4'- monophosphoryl lipid A adsorbed on aluminium hydroxide) [145]</td>
</tr>
<tr>
<td>FENDrix™</td>
<td>GSK Biologicals</td>
<td>Recombinant vaccine containing the surface antigen (HBsAg)</td>
<td>Aluminium hydroxide [162]</td>
</tr>
<tr>
<td>Recombivax HB®</td>
<td>Merck &amp; Co</td>
<td>Recombinant vaccine containing the surface antigen (HBsAg)</td>
<td>AS04 (3-O-desacyl-4'- monophosphoryl lipid A adsorbed on aluminium phosphate) [8]</td>
</tr>
<tr>
<td>Engerix™</td>
<td>GSK Biologicals</td>
<td>Recombinant vaccine containing the surface antigen (HBsAg)</td>
<td></td>
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</tbody>
</table>

The information given in this table is not meant to be exhaustive.
specific ligand activates a signalling pathway which results in cytokine secretion, up-regulation of co-stimulatory receptors and induction of host immune and inflammatory responses [124,125]. One of these TLR activating molecules, 3-O-desacyl-4′-monophosphoryl lipid A (MPL), is a derivative of a lipopolysaccharide (LPS) from the Re595 strain of Salmonella minnesota [126,127] and acts on the immune system as a TLR4 agonist [128-131]. MPL is capable of directly activating key innate immune mechanisms, including the activation of antigen-presenting cells and the induction of cytokines, such as TNFa and IL-6, which will ultimately enhance the adaptive immune response, i.e. induce T helper cells and B cell responses [132-134]. Activation of TLR4 by MPL stimulates the maturation of APCs and their migration to the lymph nodes. Thus, a higher amount of antigen is presented more efficiently to the B and T cells of the adaptive immune system resulting in an enhanced immune response to the vaccine antigen (Figure 2) [122,123,131,135].

The AS04 Adjuvant System consists of MPL either adsorbed on aluminium hydroxide for the HPV vaccine or aluminium phosphate for the HBV vaccine [136].

Preclinical data with AS04

Several immunogenicity studies performed in mice, guinea pigs and monkeys have shown the effectiveness of MPL to potentiate both specific antibody and cellular immune responses after vaccination [127,137]. Studies in mice showed that recombinant yeast-derived HBsAg adjuvanted with AS04 was able to induce an overall increase in antibody titres compared to the classical adjuvantation with aluminium alone, both in young and elderly animals [12].

The immunogenicity of AS04-adjuvanted HPV vaccine was investigated in mice and monkeys and AS04 formulation showed significantly higher titres of HPV-specific antibodies than aluminium salt formulation. In order to evaluate the quality of the humoral response, specific HPV neutralizing antibodies were analyzed in monkeys. In these studies, compared to the same vaccine antigens formulated with aluminium hydroxide alone, the formulation with AS04 induced consistently higher antibody levels throughout the observation period and elicited also a higher frequency of HPV-16 and HPV-18 L1 VLP-specific memory B cells (2.2–5.2-fold) [10]. These preclinical data provided necessary supportive evidence to evaluate the AS04-adjuvanted formulations in clinical studies for both vaccines.

AS04 mode of action

Experiments in vivo and in vitro were performed to understand where and how AS04 interacts with the immune system and which types of immune cells are involved [138]. AS04 has to be administered at the same site as the antigen at the same time or within 24 hours to trigger a transient immune response localized to the injection site and the draining lymph nodes. During this period production of specific cytokines is increased leading to higher numbers of dendritic cells and monocytes acting as antigen presenting cells in the draining lymph nodes. In the draining lymph node increased activation of antigen-specific T-cells is observed. There was no evidence of direct stimulation of adaptive immune response cells by AS04, thus making a non-antigen specific immune response very unlikely. With the exception of the site of injection and the draining lymph node there was no evidence of systemic activation of other lymphatic organs such as the spleen or distant lymph nodes, confirming that the effect of the adjuvant is localised and transient. The results of the mode of action experiments are useful to explain the clinical immunogenicity and safety profile of the vaccine, as discussed below.

AS04-adjuvanted HPV-16/18 vaccine

Immunogenicity: In Phase II studies conducted in humans, the AS04-adjuvanted HPV-16/18 vaccine was shown to induce a higher and sustained anti-HPV-16/18 neutralising antibody response along with a higher frequency of memory B cells as compared to the same vaccine antigens formulated with aluminium salts [10]. Administration of the vaccine to HPV-naïve women aged 15 to 25 years elicited high and sustained persistence of neutralising antibody titres, with > 98% of women still seropositive up to 6.4 years after the first dose [139]. According to a mathematical modelling, the vaccine is predicted to generate sustained longevity of anti-HPV-16 and -18 antibody titres, both remaining substantially above those associated with natural infection and lasting for several decades in young women aged 15–35 years [140]. Available immunogenicity data up to 8.4 years are in line with published data on antibody persistence [141]. In women 15 to 55 years of age who received three doses of AS04-adjuvanted vaccine, the levels of anti-HPV-16 and -18 antibodies in serum were shown to highly correlate with those in cervicovaginal secretions at 18 months after the third vaccine dose. This indicate that parental administration of the AS04-adjuvanted vaccine can induce serum IgG antibodies that transudate to the cervical epithelium in women at all age [142].

In a comparative study between the AS04-adjuvanted HPV-16/18 vaccine and the quadrivalent HPV-6/11/16/18 vaccine adjuvanted with amorphous aluminium hydroxyphosphate sulfate salt, the AS04-adjuvanted HPV-16/18 vaccine induced significantly higher serum neutralizing antibody titres for HPV-16/18 after the three-dose vaccination course (p < 0.0001). Positivity rates for anti-HPV-16 and -18 neutralizing antibodies in cervicovaginal secretions and circulating HPV-16 and -18 specific memory B-cell frequencies were also higher after vaccination with the AS04-adjuvanted HPV-16/18 vaccine [143].

Efficacy: In a Phase IIb study, the HPV-16/18 AS04-adjuvanted vaccine was shown to be highly efficacious against persistent infections
and pre-cancerous lesions associated with HPV-16/18. Cross-protective efficacy against incident infection with oncogenic HPV-45/31 was also observed [139]. The primary analysis of a large Phase III study in women 15 to 25 years of age confirmed the high efficacy against CIN2 or greater (CIN2+) associated with HPV types 16 and 18, which was up to 98% in an HPV naive cohort (HPV seronegative and DNA negative for oncogenic types) which approximates young adolescents before sexual debut [144]. The end of study analysis revealed that overall vaccine efficacy on CIN2+, irrespective of HPV type, was 64.9% [CI: 52.7 to 74.2] in this cohort. The corresponding value against CIN3+ was 93.2% [CI: 78.9 to 98.7] [145]. Cross-protection was observed against virological and/or histopathological endpoints for HPV-31, HPV-33 and HPV-45 [146].

Currently no studies are available where the efficacy of the AS04-adjuvanted HPV-16/18 vaccine and the HPV-6-11-16-18 vaccine are compared. Whether the differences observed in the immune profile of the two vaccines have an influence on the magnitude of protection in the long-term still remains to be determined.

**AS04-adjuvanted HBV vaccine**

The immunogenicity and safety of the AS04-adjuvanted HBV vaccine was assessed in haemodialysis patients and compared with double dose of a licensed aluminium adjuvanted HBV vaccine. In a study performed with haemodialysis and pre-haemodialysis patients aged more than 15 years, who were naive for HBV infection markers and with a documented creatinine clearance of ≤30 ml/min, it was shown that 91% of subjects who received the AS04-adjuvanted HBV vaccine (one dose containing 20 µg HBSAg administered at Month 0, 1, 2 and 6) and 84% in the control group (two doses containing 20 µg HBSAg each administered at Month 0, 1, 2 and 6) were seroprotected at Month 7. From month 1 to month 6, a significantly faster onset of protection with antibody concentrations of ≥10 mIU/ml was observed in the AS04-adjuvanted HBV vaccine group as compared to the control group. The percentage of subjects with antibody concentrations ≥100 mIU/ml was greater at all time points in the AS04-adjuvanted HBV vaccine group. At month 7, as already observed in healthy subjects, anti-HBs geometric mean concentrations were much higher in the AS04-adjuvanted HBV vaccine group (3559 mIU/ml [95% CI: 2130; 5847]) than in the control group (933 mIU/ml [95% CI: 516; 1688]) [11].

In an extension study, unselected patients were followed up to 42 months post vaccination. At Month 36 about 80% of the subjects in the AS04-adjuvanted HBV vaccine group were still seroprotected against HBV as compared with 51% in the control group. Similarly, at Month 42 about 78% of the subjects in the AS04-adjuvanted HBV vaccine group were still seroprotected against HBV as compared with 52% in the control group. These long term follow up data are consistent with the observation that pre-haemodialysis and haemodialysis patients loose more rapidly seroprotective levels of anti-HBs [147]. Therefore anti-HBs antibody concentrations have to be followed up regularly and most subjects need repeated booster doses of the vaccine. The decrease in seroprotection rate over time was significantly slower in the AS04-adjuvanted HBV vaccine group, and a booster dose was required less rapidly than in the standard HBV vaccine group. Indeed, fewer patients primed with AS04-adjuvanted HBV vaccine had to receive a booster dose during the follow-up period (16.7% versus 42.9 in control group; p=0.0098) [147].

The aim for both AS04-adjuvanted vaccines is to elicit an enhanced immune response with a long duration for their specific population. The benefits of the AS04 Adjuvant System for both vaccines are summarised in Table 4. The existence of an established serological correlate of protection for HBV allows the evaluation on how the enhanced immune response impacts the protection afforded by the HBV vaccine formulated with AS04.

### Safety of AS04-Adjuvanted Vaccines

Various preclinical toxicology evaluations did not indicate other findings for AS04 than local inflammation [12]. The safety of the AS04 has been evaluated in humans for more than 15 years. The AS04-based vaccines are generally well tolerated when evaluated during clinical development programmes. Clinical studies with AS04-based HPV and HBV vaccines showed a satisfactory and clinically acceptable reactogenicity and safety profile comparable to classic aluminium-adjuvanted vaccines [8,148,149]. The pooled safety analysis of AS04-adjuvanted HPV vaccines [149] showed no clinically relevant differences between both study groups for serious adverse events, pregnancy outcomes, and most solicited general symptoms (with the exception of myalgia, which was slightly higher in the AS04 group). Solicited local symptoms such as pain, redness, or swelling were more often reported for the AS04-adjuvanted HPV vaccine than compared with aluminium adjuvanted control vaccines. The increased local reactogenicity did not impact on vaccine compliance and this observation is in line with the experience with other adjuvanted vaccines. Since AS04 is a novel adjuvant with effects on the immune system, specific attention was given to adverse events of potential auto-immune origin in the clinical trials with AS04 containing vaccines. A large meta-analysis including all randomized and controlled studies with registered and candidate vaccines containing AS04 as Adjuvant System was performed. The integrated safety review based on more than 68,000 subjects for events of potential autoimmune aetiology revealed no statistically significant differences between the AS04-adjuvanted vaccine group and the control group with an observed event rate of approximately 0.5% for both study groups [150]. Post-marketing experience of more than 25 million AS04-adjuvanted vaccine doses distributed has confirmed an acceptable safety profile [151].

### Other Strategies for Development of HPV and HBV Vaccines

**HPV vaccines**

Currently available HPV prophylactic vaccines contain VLP from oncogenic types HPV-16/18, the two most prevalent types in

<table>
<thead>
<tr>
<th>HPV</th>
<th>HBV</th>
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<tbody>
<tr>
<td>High and sustained antibody titres in serum correlate with high antibody titres in mucosa</td>
<td>Enhanced protection of high risk population</td>
</tr>
<tr>
<td>Sustained protection</td>
<td>Longer persistence of high antibody levels</td>
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<tr>
<td>Cross-protection</td>
<td>Less need for a booster</td>
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<tr>
<td>High neutralizing levels of antibody</td>
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<td>Higher frequency of B-cell memory</td>
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<tr>
<td>Antibody persistence</td>
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<tr>
<td>Contributes to achieve needed immune profile to ensure protection</td>
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Table 4: Benefits of AS04 for the vaccines.
cervical cancer, which cause approximately 70% of cervical cancer. One strategy for improving the coverage of the vaccine against HPV-related disease is to increase the number of HPV types included in the vaccine formulation. Accordingly, a multivalent HPV vaccine with L1 VLP types 16, 18, 31, 33, 35, 45, 52, and 58 is undergoing clinical development [152]. Increasing the number of types in the vaccine however faces its own challenges, such as the potential for immune interference between the different types, as previously reported with many other vaccines [153]. Another approach under consideration is based on the L2 protein, which has the potential to generate broad protection through cross-protection [154]. Whether multivalent or L2 based vaccines will further increase the overall efficacy of the AS04 adjuvanted HPV vaccine against CIN3+ needs to be evaluated in the coming years.

Prophylactic vaccines have the potential to protect against new infections, but do not impact the progress of existing HPV infections or lesions. As a result, a therapeutic HPV vaccine would be warranted. Such a vaccine may reduce the incidence of cervical cancer by clearing early and/or late state infections by activating cellular immune responses in order to kill cancer cells which express foreign proteins, like the E6 and E7 HPV proteins.

Therapeutic HPV vaccines under development use technologies like protein vaccines or viral vectors [154]. A study with synthetic oncoproteins E6 and E7 of HPV-16 revealed that the vaccine was effective over a period of 12 to 24 months. A regression of HPV16 positive and high-grade vulvar intraepithelial neoplasia lesions in the majority of subjects was observed [155]. Another study vaccine for CIN3 immunotherapy consisting of a fusion heat shock protein covalently linked to the entire sequence of HPV 16 E7 showed efficacy in subjects infected with HPV types other than 16, suggesting cross-reactivity [156]. A clinical trial with a modified recombinant vaccinia virus expressing E2 for the treatment of CIN2 and CIN3 lesions associated with oncogenic papillomavirus showed the the vaccine was very effective in stimulating the immune system against papillomavirus resulting in a regression of high-grade lesion [157].

HBV vaccines

Vaccines based on the preS/S epitope may elicit a rapid and higher seroprotection rate compared with the conventional HBsAg vaccines in risk groups [158]. Clinical trials with an HBV vaccine adjuvanted with immunostimulatory sequences containing repeating sequences of cytosine phosphoguanosine (CpG) dinucleotide motifs have shown high titres and sustained seroprotection in healthy and hyporesponsive populations [159]. Therapeutic HBV vaccines to target and induce HBV-specific T cell responses are another area of research currently being pursued [160].

Learnings for Future Developments

The current AS04 experience with these and other vaccines is showing new opportunities in vaccine development to address unmet medical needs. The Adjuvant System approach will play a prominent role for GSK Bio in the development of future vaccines. The experiences of GSK Bio collected during preclinical and clinical studies of AS04-adjuvanted vaccines can be summarised as follows:

1) Enhanced immune response needs to be translated in clinical benefits. This can easily be shown if an immune correlate of protection is available.

2) The presence of an adjuvant in the vaccine formulation needs to be justified by scientific data suggesting that the addition of an adjuvant may result in a clinical benefit.

3) Stronger innate immune stimulation is usually accompanied with increased reactogenicity, which however needs to remain within an acceptable range.

4) Safety concerns about triggering autoimmune diseases needs to be constantly addressed during preclinical and clinical development, and post licensure surveillance.

5) Mode of action studies of the used adjuvant are becoming more and more relevant to support the observed immunogenicity and safety profile of clinical studies.

6) The long-term benefits of enhanced immune response in term of clinical protection remains to be determined.

Conclusions

Adjuvant Systems have been developed in an effort to design new vaccines that would enable the induction of enhanced immune responses aiming to increased protection, while providing a clinically acceptable safety profile.

The selection of a vaccine formulation should be based on the characteristics of the pathogen, the target population and the medical need. The Adjuvant System technology is an additional option for the development of new vaccines against challenging diseases or for subjects with immune-deficient conditions where classical approaches have proven to be less effective. There are important differences for challenging diseases like HPV and HBV to consider, but AS04 has been shown to induce enhanced immune responses.

Experience based on the AS04-adjuvanted HPV-16/18 vaccine shows that the vaccine induced enhanced and sustained levels of neutralizing antibodies against HPV, which have been shown to transudate to the cervical mucosa, along with high cell-mediated immune response.

Experience with the AS04-adjuvanted hepatitis B vaccine in prehaemodialysis and haemodialysis patients has shown that the vaccine induced higher levels of specific antibodies and increased the percentage of subjects seroprotected after vaccination. In addition, the antibody decline over time was significantly lower in the AS04-adjuvanted vaccine group, resulting in fewer booster doses needed compared to the standard vaccines adjuvanted with aluminium salts only.

In conclusion, our experience with AS04 adjuvanted vaccines encourages further evaluation of other adjuvanted vaccines for currently unmet medical needs.

Literature Search

Data for this review were identified by searches of PubMed and references from relevant articles. The following search terms were used: "human papillomavirus", "HPV", "hepatitis B", "HBV", "adjuvants", "aluminium", "MPL", "AS04", "AAHS", "immune response", "innate immune response", "adaptive immune response", "long term protection", "B cell", "memory B cell". Only articles in English were reviewed. No date restrictions were set in these searches.

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Conflict of Interest

Nathalie Garcon, Dominique Descamps, Maarten Leyssen, Michel Stoffel and Alberta Diaz Pasquale declare they are employees of GSK Biologicals.

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