



Vaccines against rotavirus and human papillomavirus (HPV)

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Abstract

Objective: To briefly review strategies aimed at the development of rotavirus and HPV vaccines, with emphasis on the current status of studies assessing the safety, reactogenicity, immunogenicity and efficacy of recently developed vaccines.

Sources of data: This review focuses on articles published from 1996 to 2006, mainly those from the last five years, with special emphasis on data obtained from recently completed studies involving a new live attenuated human rotavirus vaccine and a virus-like particle (HPV) vaccine.

Summary of the findings: Strategies for developing rotavirus vaccines ranged from Jennerian approaches to the new human-derived rotavirus vaccine. Currently, two rotavirus vaccines are recognized as both efficacious and safe: a pentavalent human-bovine reassortant vaccine and a vaccine derived from an attenuated rotavirus of human origin. The second of these has been evaluated in more than 70,000 infants all over the world. Prophylactic vaccines against HPV have been tested in more than 25,000 young individuals around the world. Results from phase II and III clinical studies indicate that such vaccines against the most common types of HPV, those linked to both genital warts and 70% of cervical cancers, are safe and highly efficacious.

Conclusions: A future rotavirus immunization program covering 60 to 80% of infants worldwide is likely to reduce by at least 50% the number of rotavirus-associated hospitalizations and deaths. It is also reasonable to expect that implementation of HPV prophylactic vaccines will reduce the burden of the HPV-related diseases that presently impact millions of people around the world.

J Pediatr (Rio J). 2006;82(3 Suppl):S25-34. Rotavirus, gastroenteritis, vaccine, human papillomavirus, HPV, cervical cancer, genital warts.

Introduction

The worldwide impact of diseases caused by rotavirus is extremely significant, both in developed countries and developing ones.¹ There is, however, a marked difference if these two panoramas are compared: whereas deaths are rare in the first category, in the poorer regions of the planet approximately half a million children die each year infected by rotavirus. Recent estimates convert this impact into 111 million episodes of diarrhea each year, 2 million of which require hospitalization and result in the deaths of at least 600,000 children aged less than 5 years.²

During the last two decades, the development of an effective vaccine against rotavirus won well-publicized priority in the programs of several international

organizations, including the World Health Organization (OMS), the Institute of Medicine in the United States and the Global Alliance for Vaccines and Immunization.³ Multiple strategies resulted, leading from the pioneering Jennerian approach right up to the advent of vaccines now in phase III of trials or even to licensure of vaccines. The information that follows is a synthesis of the strategies adopted to date, with emphasis given to vaccines currently in testing or in the process of being licensed.

Pioneering strategies: brief historical background

Jennerian procedures

The first candidates for rotavirus vaccines were identified using a similar strategy to that used successfully by Edward Jenner more than two centuries ago. The pioneering experiments used the RIT 4237 and WC3 samples, both of bovine origin, which were tested in Finland and the USA, respectively. Although the efficacy levels achieved in those two countries were significant (> 80%), subsequent investigations in South America and Africa demonstrated frustratingly low protection, resulting in the abandonment of trials.⁴

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Next, several studies were undertaken using a viral sample of simian origin, RRV (or MMU 18006), serotype G3, with highly variable results. At this point, the theory that type-specific protection might be required led to the development of polyvalent preparations.

Modified Jennerian strategies

These strategies resulted in genetically restructured samples, taking advantage of the segmented nature of the viral genome by cocultivation of rotavirus from both animal and human origins. The proposal was to develop chimeras containing 10 rotavirus genes of animal origin and another from viral samples from humans.⁵ Such preparations are basically composed of rotavirus strains resulting from the genetic permutations between the WC3 and RRV samples and the serotypes that infect humans.

The advent of the rhesus-human reassortant tetravalent vaccine (RRV-TV), of human-simian origin, occasioned innumerable and extensive studies in many countries.^{1,6-9} The impressive level of efficacy achieved with this vaccine, in particular in Venezuela, were the basis for its license being granted in the USA in August 1998, under the trademark Rotashield® (Wyeth Laboratories®, Inc., Marietta, Pennsylvania). One year later, after it had been administered to 900,000 children, 15 cases of intussusception emerged as a possible severe adverse event associated with the vaccine, leading to its suspension.^{10,11} It was noted that 81% of these cases were in children older than 3 months, despite the fact that this age group accounted for less than half of the total vaccinated population. This evidence has resulted in recommendations for the new generation of vaccines that limit the age at administration of the first dose to 12 to 14 weeks. Nowadays there is consensus that 1 child in every 10,000 vaccinated with Rotashield® is at risk of developing intussusception.^{1,12,13}

A second group of vaccines involved preparations of bovine (WC3 sample) and human origin, resulting in a tetravalent formulation to be given in three doses. Studies of its efficacy indicated 67% protection from all rotavirus-induced diarrhea episodes.¹⁴

Vaccine candidates of human origin

These procedures were based on the observation that repeated natural infections culminate in solid protection.¹⁵ The first attempts involved rotavirus obtained from newborn infants in wards. The M37 sample, G1 type, isolated from a neonate in Venezuela, was the first candidate for a vaccine that resulted from these strategies. The insignificant efficacy rates achieved in tests in Finland, however, caused the discontinuation of studies with M37.¹

Current vaccine scenario: ongoing tests and licensure

The apparent failure of the tests involving the vaccine candidates described in the previous section, chiefly the suspension of Rotashield® in the USA, culminated in unexpected delay. Nevertheless, decisive lessons were learnt that contributed to the current, auspicious, moment. This situation was particularly well described by Glass et al.,¹⁶ in a recent article with the emblematic title "The future of rotavirus vaccines: a major setback leads to new opportunities." Table 1 collects, in a synthesized manner, the basic characteristics of vaccines that are currently on trial or already licensed.

Below is a brief description of the characteristics of each vaccine in the current generation, undergoing clinical trials or duly licensed, with emphasis on RotaRix®, which was recently licensed in some Latin American countries.

LLR

This is a vaccine represented by attenuated rotavirus of ovine origin, serotype G10, produced by the Lanzhou Institute of Biological Products in China. Although it is licensed for wide-scale use in that country, reservations persist about the methodology employed during testing.^{1,4}

RotaTeq®

This is a pentavalent vaccine preparation derived from the WC3 bovine sample, combining samples genetically restructured to be specific for G1, G2, G3, G4 and P1A[8]. This is a non-reactogenic product with elevated efficacy, achieving 100% protection against the most severe episodes of diarrhea.¹⁷ Recently, phase III trials of RotaTeq® were completed, involving at least 68,000 children, and, at the start of 2006, the vaccine was licensed by the Food and Drug Administration (FDA) in the USA. In addition to being free from intussusception risk, the vaccine has also proved itself 94.5% effective against hospitalizations and emergency consultations related to the G1 to G4 viral types, reduced gastroenteritis associated with these serotypes by 74% and demonstrated 98% protection from severe episodes caused by rotavirus.¹⁸

RotaRix® (RIX4414 sample)

Worthy of special attention, among strategies involving attenuated rotavirus of human origin, is RotaRix® (RIX4414 sample) by GlaxoSmithKline® Biologicals (GSK), Rixensart, Belgium. This monovalent preparation, from the ranks of the multiple candidates for rotavirus vaccines, is now at an advanced stage of trials and has already been licensed in some South American countries and in Kuwait.⁴ Testing of RIX4414 involved at least 72,000 children in 20 countries,

Table 1 - Vaccines against rotavirus at varying phases of analysis or already licensed

Vaccine	Producer	Origin and preparation characteristics	Development stage
LLR	Lanzhou Institute of Biological Products	Ovine, type G10	Licensed in China
RotaTeq®	Merck Sharp & Dohme® (USA)	Bovine-human, pentavalent	Phase III complete and licensed in the USA
RotaRix®	GlaxoSmithKline® (Belgium)	Human, monovalent	Phase III concluded and licensed in some countries *
UK derivative	National Institutes of Health (USA)	Bovine-human, tetravalent	Phase II
RV3	University of Melbourne (Australia)	Neonatal, monovalent	Phase II
116E and I321	Bharat Biotech® (India)	Neonatal, monovalent	Phase I
RRV-TV	NIH and Biovirx® (USA)	Simian-human, tetravalent	Licensed in the USA, no longer produced

* Mexico, Dominican Republic and Brazil.

in at least 15 different clinical trials. The results of these multiple analyses are described very briefly below.

Origin of the vaccine and precursor studies

The viral sample (originally designated 89-12) was isolated from the feces of a child in Cincinnati, Ohio, USA, who had moderate diarrhea.¹⁹⁻²¹ Passage through cell cultures and cloning procedures resulted in the attenuated RIX4414 sample, specific for G1 (glycoprotein) and P[8] (protease sensitive protein).²¹ The product currently in licensing is for oral use and is presented lyophilized in individual flasks with administration indicated between 2 and 4 months of age.

Pre-clinical testing of RIX4414 was undertaken in Finland, with aspects related to safety, immunogenicity and efficacy being investigated.²² No significant side effects were observed and satisfactory immunoresponse was observed in 95% of susceptible children.²³ On the other hand, for clinically severe diarrhea episodes, protection was calculated as being in the order of 90%.²⁴

Extensive phase II trials of the RIX4414 vaccine

The promising results from Finland laid the foundations for the development of further studies in Latin America (Brazil, Mexico and Venezuela) and Singapore.^{22,25-27} The trials carried out in Latin America involved 2,155 children, who were given two doses of the vaccine, assessed as

three distinct concentrations [$10^{4.7}$ plaque forming units (PFU), $10^{5.2}$ PFU and $10^{5.8}$ PFU] or placebo, at 2 and 4 months. The trials in Asia involved 2,464 individuals who were given vaccine or placebo at 3 and 4 months. The principal indicators of reactogenicity, safety, immunogenicity and efficacy observed in Latin America are detailed below.

Tolerance to the vaccine was assessed, noting the frequency of "solicited symptoms" for 15 days after each dose was administered. Percentages of fever, diarrhea, vomiting, irritability, anorexia and coughing/coryza were comparable for all three vaccine concentrations and placebo (Figure 1).^{22,25,27} No relevant severe adverse events were observed, nor were there any deaths with causal links to vaccination, including intussusception.

Immunoresponse was also analyzed in the phase II trials in Latin America, in terms of the frequency of serological conversions expressed by immunoglobulin A specific for rotavirus. Figure 2 demonstrates a variation of 61 to 65% for the serum samples collected after the second dose, in clear contrast with the children given placebo.

On the subject of immunoresponse, it should be noted that the take of the vaccine – a combination of serological conversion and excretion of the vaccine virus – reaches 75% after two doses at the highest concentration, i.e. $10^{5.8}$ PFU.^{25,27}

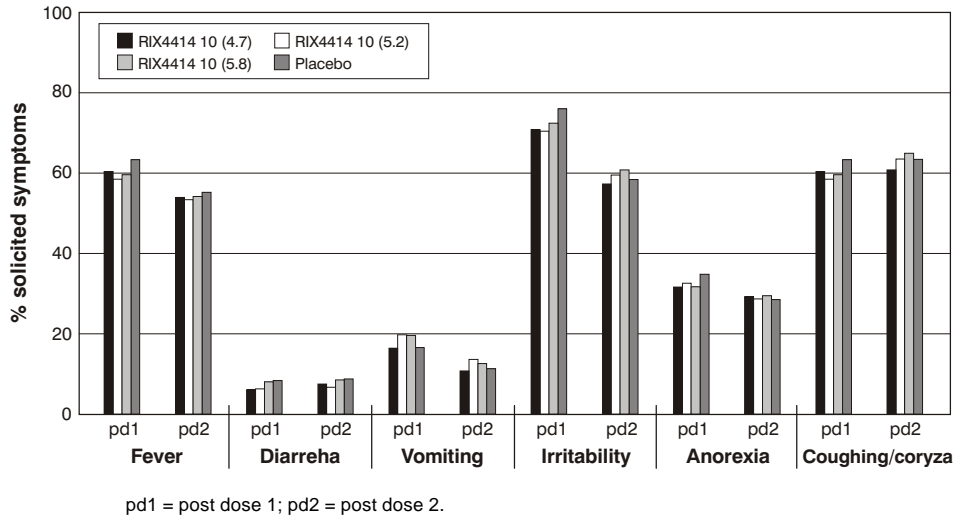


Figure 1 - Frequency of "solicited symptoms" for 15 days after administration of RIX4414 vaccine or placebo (adapted from Salinas et al.²⁷)

One well-publicized and practical aspect related to immunogenicity is the fact that when RIX4414 is administered concurrently with routine vaccines, there is no interference whatsoever with their immunoresponse.^{22,25,27} Phase II specified an interval of 15 days between the administration of RIX4414 and the oral polio vaccine (OPV). Studies of the concurrent use of both, carried out in South Africa, provide evidence that the phenomenon of interference does not exist.²⁷

In general, efficacy rates are more significant for protection against severe diarrhea episodes, based on the clinical scoring system proposed by Ruuska & Vesikari.²⁸ Figure 3 lists efficacy rates, establishing a correlation between vaccine concentration and severity of

gastroenteritis due to rotavirus. The greatest degree of protection from severe episodes was 86%, observed with the 10^{5.2} PFU or more dosage.²⁷

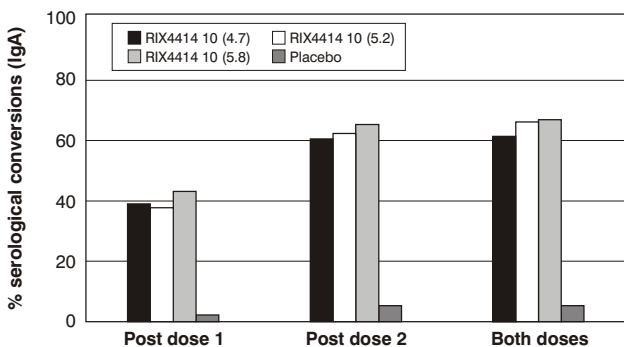


Figure 2 - Serological conversions, expressed in rotavirus specific IgA levels for children given vaccine and placebo (adapted from Salinas et al.²⁷)

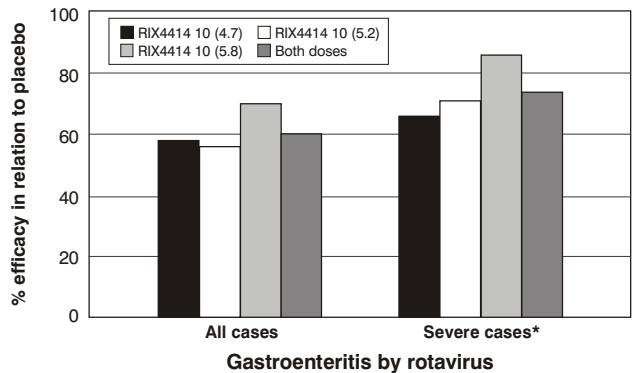


Figure 3 - Efficacy of the RIX4414 vaccine by viral concentration and clinical severity of gastroenteritis by rotavirus (adapted from Salinas et al.²⁷)

Despite the vaccine's monovalent character, heterotypic protection was also observed. In addition to the 88% efficacy for the G1 serotype, it has also become noteworthy for the protection (83%) it offers against other types in circulation, including G9, which is emerging on a global scale.^{25,27}

Extensive phase III trials, which had recruited 63,225 children, were recently concluded in 11 Latin American countries and Finland.²⁹ The impressive size of this sample made it possible to investigate the (previously) postulated risk of intussusception. The result was that, during the 31 days following each dose, 6 cases were observed among children given the vaccine and 7 among those given placebo (RR = 0.85; CI = 95%; p = 0.78). Furthermore, they confirmed the previous indications of the elevated efficacy offered by RIX4414 for severe episodes of gastroenteritis by rotavirus, reaching 85%. With respect to the serotypes covered, both homotypic (against gastroenteritis by G1) and heterotypic (G3, G4 and G9) efficacy became evident. The level of protection offered against G2, in the order of 45%, is apparently low, but closer examination reveals that this reflects the low number of samples isolated, to judge by the wide confidence interval. Indeed, a meta-analysis of phase II and III results that did have a sufficiently large number of G2 rotavirus samples, clearly demonstrated efficacy, reaching 67%.²⁹

UK bovine sample derivative

Another vaccine candidate based on genetic permutations between rotaviruses of animal and human origins is the tetravalent (G1, G2, G3 and G4) preparation derived from the UK sample, conceived by the NIH, USA. Phase II trials indicate satisfactory levels of innocuity, efficacy and immunogenicity.^{30,31}

Samples of neonatal origin (RV3, 116E and I32)

Currently appearing as monovalent rotavirus vaccine candidates are three samples isolated from newborn infants, one evaluated in Australia (RV3) and the other two in India (116E and I321). The RV3 sample has antigenic identity with the G3 viral type and is free from adverse reactions and moderately immunogenic.^{1,4} The 116E (serotype G10) and I321 (G9) samples have immunogenic potential, in addition to the efficacy they exhibited against symptomatic rotavirus reinfection.^{1,4}

RRV-TV

The North-American company Biovirx[®] is currently planning a possible return to production of Rotashield[®]. They claim that the benefits of the vaccine in developing countries considerably outweigh any possible risks represented by intussusception. Those that defend a return to RRV-TV production claim that the risks themselves were overestimated in analyses performed so far.⁴

Prospects

Although the results from the two vaccines RotaRix[®] and RotaTeq[®], based on live attenuated viruses, are

promising, efforts continue to create new preparations for probable future use. Two that stand out are a simian-human hexavalent formulation,³² and another with a rotavirus of porcine origin as a substrate to be genetically rearranged with serotypes that infect humans.^{33,34} Possible vaccines of the future contain deactivated rotavirus (or viral fragments) which is, theoretically, free of any risk whatsoever of intussusception.¹

Vaccine against human papillomavirus (HPV)

Global estimates indicate that approximately 20% of normal individuals are infected by any type of HPV. Furthermore, HPV infection causes almost 500,000 new cases of cervical cancer per year, 70% of which occur in underdeveloped or developing countries. It is also estimated that there are 10 to 20 times more precursor lesions than tumors, which implies a larger number of affected individuals.³⁵ Early diagnosis and control of these neoplasms has been based for more than 40 years on the observation of morphological alterations in cervical smears as developed by G. Papanicolaou. In countries where cervical screening has been extended to the majority of the population, a significant reduction in the incidence of and mortality by these tumors is observed. Unfortunately, less than 15% of Brazilian women are involved in a cervical cancer prevention program; this explains, in part, the high incidence of this neoplasm in our country. Nevertheless, even in developed countries, with wide coverage of their population by prevention programs, there is a significant percentage of women who continue to succumb to the disease because of Papanicolaou test failures.³⁶

There is sufficient molecular and epidemiological evidence to confirm the link between certain types of HPV and cervical cancer and its precursor lesions. Recently, several different studies indicated that detecting the DNA of high risk HPV in cell smears predicts the presence of a cervical cancer precursor lesion in patients who have negative or doubtful cytology results. In addition, there are good indications that an HPV molecular test can serve as "quality control" for cytology, reducing the number of false negative results.³⁷

More than 98% of cervical tumors are caused by these viruses.³⁶ The types present in these tumors are different from the ones found in benign lesions of the anogenital region, the most frequent of which are condylomas, particularly among young or immunodepressed patients. Another pathology that, despite being very rare, well represents the impact of HPV infections is laryngeal juvenile papillomatosis, or recurrent respiratory papillomatosis, caused by types 6 and 11, which are typically of low oncogenic risk and are rarely found in malignant tumors.³⁸

More than a hundred different HPV types have been described and approximately half of these are considered of high oncogenic risk. The most commonly observed types in malignant neoplasms of the anogenital region, in different populations worldwide, are 16, 18, 31, 33, 45, 51 and 58. Approximately half of all cervical cancer cases are caused by HPV 16, which is also involved in the genesis of other anogenital tumors, such as of the vulva, penis and anus, although in much lower proportions.³⁸

Infections by HPV are relatively common in normal individuals, varying from 20 to 40% depending on age and immune status, being more common in the young. Most of these infections are totally asymptomatic and regress spontaneously.³⁹ The risk of developing the disease is associated with persistent infections by high-oncogenic risk HPV types.⁴⁰ Therefore, any measure that controls HPV infections should have an impact on the control of pathologies linked to them. The first impact should be reflected in a reduction in the rates of precursor lesions, but the ultimate objective is to control the incidence of cervical cancer. Based on the administration of a vaccine with elevated efficacy, it could be estimated that a 75% reduction in the prevalence of type 16 HPV alone could signify a 75% drop in the incidence of cervical intraepithelial neoplasms (CIN). Thus, safe and effective vaccines against HPV could be important instruments in the prevention of cervical cancer worldwide, but particularly in developing countries.

Vaccines against animal papillomaviruses

Papillomaviruses are species specific and are not therefore transmitted between different animal species. There is, however, a high level of similarity in the genomic structure of these viruses, which makes it possible to extrapolate to humans a series of features of the virus-host relationship observed in animals, particularly with relation to the immunological aspects of these infections. Noteworthy studies have involved rabbits, dogs and cows, and the viruses cottontail rabbit papillomavirus (CRPV), canine oral papillomavirus (COPV) and the many types of bovine papillomavirus (BPV).⁴¹

At present the most effective vaccines are those that have been developed for use against CRPV and COPV. Both are capable of controlling the development of papillomas caused by the viruses on the skin and oral mucosa of rabbits and dogs, respectively, preventing its natural progress to malignant tumors, observed in a certain proportion of cases. The vaccine employing late COPV antigens in the form of virus-like particles (VLP) prevents the development of warts in the oral mucosa of 100% of dogs exposed to the virus. A similar study, using L1 late antigen from CRPV, was conducted with rabbits, and the same level of effectiveness in controlling

the appearance of papillomas on the skin of rabbits was achieved. This animal model has provided very important data on the cellular immunoresponse to these viruses, with emphasis on the role of polymorphism of the major histocompatibility complex (MHC) genes. The variation in binding and presentation of viral antigens appears to interfere in the clinical outcome of lesions associated with animal oncogenic papillomaviruses, and possibly of human ones.⁴¹

Despite the complexity of the events involved in triggering an effective immunoresponse, the studies performed with animals have provided very positive results with respect of the use of these vaccines. A series of protocols for the development and utilization of vaccines against HPV have since been generated.⁴²

HPV antigens

The papillomavirus capsid is made up of two proteins, designated L1 and L2. The expression of the late gene L1 alone, or L1 together with L2, in a wide range of expression systems (bacteria, yeasts, insect cells), generates particles with structures that are very similar to the virions isolated from natural lesions, but which do not contain viral DNA and are designated VLPs. As described above, these are the main source of antigens for animal vaccination. Given the similarity between the genetic structures of these viruses, the same strategy has been employed with success to produce HPV late antigens, resulting in the antigens that are being employed in clinical trials with humans.⁴¹

In addition to the L1 or L1 and L2 VLPs for several types of HPV, some early proteins have also been proposed as vaccine antigens, particularly E6 and E7, because they are directly involved in the uncontrolled cell proliferation and transformation. In this scenario the vaccine would have therapeutic qualities, while the first type aims at infection prophylaxis by developing an immunoresponse against viral capsids that simulate natural infections by these viruses.

Recently it proved possible to synthesize, using the systems described above, a VLP consisting of L1/L2 and one of the early proteins that exists within the viral capsid structure. These particles are known as chimeric VLPs and are very attractive vaccine antigens, since they can be used both for prophylaxis and treatment of HPV-related lesions. Experimental models with induced tumors caused by HPV 16 in mice proved their therapeutic efficacy, which led some research teams to propose their use with humans as vaccines that would be both prophylactic and therapeutic.⁴³ The efficacy of these vaccines is being tested on patients with advance cervical carcinomas. To date results indicate that there is weak specific immunoresponse to viral antigens, probably

due to the fact that the majority of these patients are immunodepressed due to the advanced stage of their disease. The knowledge that is being accumulated, however, will soon be employed in clinical trials involving patients with tumors at less advanced stages.

Current status of vaccination trials in humans with prophylactic vaccines against HPV⁴⁴

Clinical trials of prophylactic HPV-16 vaccines have been ongoing since 1997. In the majority of these trials, injections of VLP purified from yeast or insect cell cultures containing recombinant vectors expressing the HPV-16 L1 or L1 and L2 genes have been used. These expression systems are highly efficient, despite the fact that the processes involved are time consuming and relatively expensive. Nevertheless, purified VLPs are being used in double-blind, placebo controlled clinical trials at different stages around the world.

The results of phase I clinical trials of prophylactic vaccines for HPV types 11 and 16 indicate that both subcutaneous and intramuscular administration of the vaccine are safe, causing no adverse reactions or small scale reactions, such as local pain or fever for short periods, comparable to the control group that were given placebo (saline or aluminum hydroxide). The doses reported vary from 10 to 100 micrograms of purified VLP, injected pure or combined with aluminum hydroxide as an adjuvant. After the initial dose, two or three booster doses were given at intervals varying from 4 to 16 weeks. Trials reported to date state that after vaccination individuals exhibited good immunoresponse, determined by an increase in seropositivity for viral antigens specific for each type. Furthermore, neutralizing antibodies were detected at levels exceeding those observed in individuals naturally infected by HPV, indicating that, in these trials, the vaccine is immunogenic.⁴¹

The results of phase II clinical trials, designed to define the toxicity and immunogenicity of prophylactic HPV vaccines, are encouraging. Nevertheless, the efficacy of these vaccines will only be truly assessed in phase III clinical trials involving a large number of individuals representing populations at risk of exposure. It is well known that the populations at highest risk of developing cervical cancer and its precursor lesions reside in underdeveloped and developing countries, which is where the clinical trials of the efficacy of these vaccines should be carried out. In addition to the vaccines composed of VLPs of L1 from HPV-16,⁴⁵ vaccines are also being tested containing four viral antigens, corresponding to types 6, 11, 16 and 18, in randomized, double-blind, placebo controlled trials. Initial results indicate that the quadrivalent vaccine does not cause severe adverse reactions, and is well tolerated. Furthermore, the antibody levels produced

are many times greater than those resulting from natural infections by this virus. These results made it possible to define the doses that are being used in phase III clinical trials, set to demonstrate whether the various formulations are capable of controlling not just infections, but also the development of lesions caused by these HPVs in a large numbers of individuals. It should be noted that the immune responses obtained are, in essence, species-specific, i.e. they should protect against the types of HPV contained in the vaccines being tested.⁴² There is much interest, therefore, in investigating the possibility of cross-protection, given the genetic similarity between the many different types of HPV. The ongoing clinical trials could provide information that is still lacking in this matter.

Prophylactic vaccines are being tested in many centers worldwide, including in Brazil. Since the main goal is to prevent infection by those HPV types most commonly linked with benign (types 6 and 11) or malignant (types 16 and 18) lesions, healthy volunteers are being recruited. These volunteers, aged 16 to 24 years of age, should have had less than four sexual partners, to avoid prior HPV infection. The quadrivalent vaccine is being administered in three intramuscular doses in randomized, double-blind, placebo controlled clinical trials. Recently, the results were published for two prophylactic vaccines composed of HPV VLPs, the first containing two types (16 and 18),⁴⁶ and the other a quadrivalent vaccine (6, 11, 16, 18).⁴⁷ Both vaccines were shown to be safe, well tolerated by the young volunteers. Yet more important is that the immunoresponse triggered by both forms was extremely elevated, with antibody titers more than one hundred times higher than the levels observed in women of the same age group naturally exposed to the various HPVs being studied. Finally, in the two published clinical trials, the vaccines exhibited elevated efficacy, having controlled from 90 to 100% of infections by the HPV types included in the vaccines, in addition to preventing 95 to 100% of the lesions caused by these viruses. Table 2 summarizes the results obtained in a comparative manner.

Phase III clinical trials have been in progress since 2002, involving tens of thousands of volunteers, including young women (more than 20,000), middle-aged women, children, adolescents and young men, both heterosexual and homosexual. These studies are being conducted in many countries worldwide, including Brazil. The first results of one of these phase III trials were released recently. It was coordinated by Merck Sharp & Dohme® (MSD) and involved approximately 12,000 young women, half of whom were given placebo and half the L1 quadrivalent vaccine for HPV 6, 11, 16 and 18. In addition to confirming that the vaccine is safe, well tolerated and highly immunogenic, an efficacy rate of 100% was observed for the prevention of cervical cancer precursor lesions.⁴⁷ These excellent results supported the application to the

Table 2 - Results of two phase II clinical trials of prophylactic HPV vaccines

	Merck Sharp & Dohme®⁴⁷	GlaxoSmithkline®⁴⁶
Antigen and adjuvants	VLP of HPV 6, 11, 16 and 18 in aluminum hydroxide	VLP of HPV 16 and 18 in aluminum hydroxide and MPL
Study population	552 women aged 16 to 23 years, with up to four sexual partners in life	1,113 women aged 15 to 24 years, with up to six sexual partners in life
Inclusion criteria	Included HPV positive women	Included just HPV negative women
Follow-up duration	36 months	27 months
Efficacy in the population, according to protocol *		
Persistent infection †	89%	100%
Cervical lesions ‡	100%	95% §
Genital warts	100%	NA

NA = not available.

* Seronegative women also negative for DNA of vaccine HPV types

† HPV 16 and 18.

‡ Includes degrees I, II and III cervical intraepithelial neoplasms (CIN).

§ Based on cytology results only, while in the MSD trial there was histopathological confirmation and exit colposcopy of all participants.

various regulatory authorities in many different countries for the vaccine to be licensed; in the USA, the FDA estimates that by June 2006 the product will have been cleared for sale. This means that, in 2006 or 2007, the first quadrivalent vaccine against HPV (GARDASIL®, MSD) will be commercially available and will begin to be used in the USA and other countries that approve it.

Final comments

The primary goals of an international program for the development of vaccines against rotavirus specify an immunization program covering 60 to 80% of the world's children in the next 10 years. Achieving this, it is estimated that the number of rotavirus related deaths and hospitalizations would be reduced by 50 to 60% globally.^{3,4} Notwithstanding, although there now exist real chances of achieving this objective, there are still obstacles to be overcome. While the two vaccines in the most advanced stages of development (RotaRix® and RotaTeq®) offer excellent performance, studies have not yet been carried out in regions where extreme poverty is prevalent (for example, Africa and Asia), in order to consolidate their claims to universal efficacy. In these communities factors like malnutrition and enteroviral and bacterial infections act as determinants of potential interference.¹ Likewise, there is also reservation about the use of live attenuated rotaviruses with immunodepressed individuals (those

infected by HIV, for example), which is an issue that requires urgent investigation. A myriad of other questions emerge with clarity as we stand on the temporal threshold of an era when at least two vaccines against rotavirus exist. For the health ministries of developing countries, the costs and sustainability of any proposed program for universal immunization against rotavirus remain central issues. Indeed, credit is due to the Health Ministry in Brazil for the pioneering role they have taken, guaranteeing widespread access to the rotavirus vaccine in the public system in 2006.³⁴ Other relevant challenges are to study the economic impact, possible technology transfer to certain countries for "regionalized" vaccine production and educational programs aimed at achieving complete awareness of the importance of immunization. In parallel, the expansion of systematic vigilance networks becomes indispensable. These will provide trustworthy and representative data on the real impact of rotaviruses and of the vaccine when made available to the general population.

In relation to prophylactic HPV vaccines, given the difficulties in implementing screening programs, which, in addition to technical and political issues, come up against sociocultural and behavioral issues, we believe that vaccines with elevated efficacy against HPV could, over the medium and long term, have a real and more significant impact on cervical cancer rates (and also rates of pre-malignant lesions), which, in Brazil, remain

extremely high. Since HPV is a sexually transmitted infection, prophylactic vaccines should be given prior to first coitus. This implies vaccinating children/adolescents of both sexes. It is also reasonable to consider the vaccination of pregnant women, but these studies have not yet been initiated. Nevertheless, the ideal age group for vaccination depends on a series of factors, including the length of protection (longevity of the immunoresponse), which is still being evaluated by ongoing clinical trials (the longest follow-up period is 4 years). It is reasonable to suppose that through a progressive implementation of vaccination against HPV, changes will be observed in the frequency with which Papanicolaou tests are needed. The expectation over the next few decades is that the prevention of cervical cancer will continue to be based on periodic screening of the population using the Papanicolaou test, in isolation or with molecular testing for HPV, the etiologic agent of these tumors. In conclusion, safe and effective vaccines against HPV could become important instruments for the prevention of cervical cancer globally, particularly in developing countries. The expectation is that in 10 to 20 years we may observe reductions in the incidence of precursor lesions of this cancer and, by degrees, reductions in the cancer that is the second largest cause of death in women due to neoplasms worldwide.

Conflict of interest

Alexandre C. Linhares declares that he has delivered lectures on the rotavirus vaccine at national symposia promoted by GlaxoSmithKline Biologicals (GSK), Rixensart, Belgium. Furthermore, he was involved, in the role of principal investigator, in the phase II and phase III trials of the RIX4414 sample (brand name: Rotarix), carrying out what was detailed in the specific protocols. In order to further these studies, the Instituto Evandro Chagas, SVS, Health Ministry, in accordance with a financing agreement with GSK and a local foundation, received finances solely destined to pay for external personnel, supplies, third-party services and certain equipment. Luisa Lina Villa declares that she is a Merck, Sharp & Dohme consultant and has delivered several lectures on her clinical problem involving the quadrivalent HPV vaccine (Gardasil).

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