

## RECENT ADVANCES – VACCINOLOGY IN CHILDREN

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**Abstract :** The field of vaccinology is highly dynamic. Newer vaccines as well as vaccine technologies are constantly being developed. Novel technologies such as chimerivax and virosomes have led to more efficacious vaccines. Combination vaccines and ready to use preparations offer better compliance and ease of administration. Newer vaccine adjuvants lead to increased vaccine immunogenicity. Vaccines against diseases with significant morbidity and mortality such as rota virus diarrhoea, viral hepatitis A, cervical cancer due to human papilloma virus, Japanese encephalitis, conjugated pneumococcal and conjugated typhoid vaccines have recently been approved. Lastly, vaccines under development such as those against malaria, dengue and HIV are in various stages of development. Future in vaccinology lies in the development of DNA vaccines, therapeutic vaccines and vaccines against non-infectious diseases such as malignancies and autoimmune disorders. The following review details recent updates in the field of vaccinology with special reference to the pediatric population

### INTRODUCTION

For many years people have noted that prior contact protected against future infections. Because of the enormous development in the field of immunologic research, most children today receive protection from the common childhood illnesses in the form of “vaccines”. The science of vaccines is a highly dynamic and cost-effective branch with a need to update oneself constantly on the recent developments in the subject. This review details recent updates in vaccine technology, newer vaccines and the future of vaccinology.

### NEWER VACCINE TECHNOLOGIES

#### CHIMERIVAX TECHNOLOGY

This is a novel approach for rapid development of a molecularly-defined, live, attenuated vaccines. The technology (ChimeriVax) is applicable to the development of vaccines against flaviviruses, and products against Japanese encephalitis, West Nile and dengue are undergoing clinical trials, respectively. ChimeriVax vaccines utilize the safe and effective vaccine against the prototype flavivirus -yellow fever 17D- as a live vector. Infectious clone technology is used to replace the genes encoding the pre-membrane (prM) and envelope (E) protein of yellow fever 17D vaccine with the corresponding genes of the target virus (e.g. West Nile). The resulting chimeric virus contains the antigens responsible for protection against the target virus but retains the replication efficiency of yellow fever 17D<sup>1</sup>.

#### VIROSOMAL TECHNOLOGY

This novel technology has been used in developing vaccines against hepatitis A, influenza and malaria. Out of these the virosomal hepatitis A vaccine is commercially available. The hepatitis A virosomes are spherical vesicles made of unilamellar phospholipid bilayers atleast 100 times smaller than the particles in aluminium adjuvanted vaccines. Purified haemagglutinin (HA) and neuraminidase (NA) influenza surface glycoproteins, isolated from the influenza A/Singapore 6/86 (H1N1) virus strain, are intercalated into this phospholipid bilayer. The final step in the production of the virosomes is the adsorption of the formalin-inactivated, highly purified HAV virions of the RG-SB strain onto the virosome surface<sup>2,3</sup>.

It has been shown that the influenza virus HA component of the virosome enables binding to immunocomponent cells such as macrophages leading to endocytosis. Within the endosome, the virus

antigen is proteolysed to antigenic peptides. Thereafter, the antigen containing endosomes join with major histocompatibility class II (MHC II) molecules. The resulting MHC II-antigen complex is transported to the cell surface where it initiates both humoral and cell-mediated immune response. This natural process of antigen presentation enables effective HAV antigen processing and the stimulation of protective immune responses without inducing the non-specific inflammatory response characteristic of aluminium-salt based vaccines<sup>3</sup>.

### COMBINATION VACCINES

With the introduction of increasing number of newer vaccines to prevent childhood diseases combination vaccines are a solution to the problem of increased number of injections during hospital visits. A combination vaccine consists of two or more immunogens combined in a single preparation. This is in contrast to simultaneous vaccination in which there are concurrent but physically separate injections. Combination vaccines commonly used are diphtheria and tetanus alone (DT or dT) or with pertussis vaccine (DPT), inactivated polio vaccine (IPV) and MMR vaccine. Newer penta or hexavalent vaccines also include HiB and/or hepatitis B in the above combination. The advantages of combination vaccines include reduced number of injections with consequent reduction in parental anxiety, pain to the vaccinee and risk of needle stick injury to the vaccinator. There is higher compliance, reduced number of visits, reduced need for storage space, packaging, handling, transportation, less documentation and logistics.

Some of the *drawbacks* of combination vaccines include chemical interference and immunologic interference between the constituent antigens. Consequently, vaccines should not be combined at the time of administration and only authorised combination vaccine used.

### READY TO USE PREPARATIONS

Many vaccines are now available in single-dose pre-filled syringes thus eliminating the need for reconstitution and hence causing minimal contamination and risk of infection.

### RECENT ADVANCES IN VACCINE ADJUVANTS

Adjuvants are substances that boost the immunogenicity of vaccines. Adjuvants can be broadly separated into two classes based on their

principal mechanisms of action: vaccine delivery systems and immunostimulatory adjuvants. Vaccine-delivery systems generally are particulate (e.g., emulsions, microparticles, iscoms, and liposomes) and function mainly to target associated antigens into antigen-presenting cells. In contrast, immunostimulatory adjuvants are derived predominantly from pathogens and often represent pathogen-associated molecular patterns (e.g., lipopolysaccharide, monophosphoryl lipid A, CpGDNA) which activate cells of the innate immune system. The discovery of more potent adjuvants may allow the development of prophylactic and therapeutic vaccines against cancers and chronic infectious diseases. In addition, new adjuvants may also allow vaccines to be delivered mucosally<sup>4</sup>.

## NEWER VACCINES

### *Rotavirus vaccine*

Globally, rotavirus is the single most important etiologic agent of severe diarrhea in infants and young children, with 114 million cases, 25-55% of all hospital admissions for diarrhea and more than 610,000 deaths per year<sup>5,6</sup>. In India, 6-45% of all childhood diarrhea requiring hospitalisation is due to rotavirus. Serotypes G1, G2, G3, P8, P6 and P4 account for 65-70% of rotavirus infections in India<sup>7</sup>. It has been reported that, after the first rotavirus infection, 88% children are protected against severe gastroenteritis and following a second infection, virtually all children are protected against severe diarrhea and most are protected against any rotavirus disease<sup>8</sup>. Hence an attenuated vaccine that simulates natural infection, and is administered in two doses, should induce protective immunity and prevent severe diarrhea and its complications<sup>9</sup>. Rotavirus diarrhea causes significant mortality and morbidity. Its consequences are more severe in the underprivileged. Moreover, in developed countries it was observed that even with improved sanitation the prevalence of rotavirus diarrhea did not decrease. Hence the vaccine will be a useful addition to the national immunisation programme.

Currently, two live viral oral vaccines are licensed and marketed worldwide, Rotarix<sup>TM</sup> and Rotateq<sup>TM</sup>. Rotarix<sup>TM</sup> is a monovalent attenuated human rotavirus vaccine derived from the strains 89-12. Rotateq<sup>TM</sup> is a human bovine reassortant vaccine. Large phase 3 double-blind placebo controlled trials from USA, Europe and Latin America have shown 85-98% efficacy against severe rotavirus gastroenteritis and 42-59% efficacy against hospitalisation due to diarrhea of any cause. Both vaccines are safe with no increased risk of intussusception<sup>10, 11, 12</sup>. Studies show no interference between rotavirus vaccines and other childhood vaccines including IPV, pneumococcal, Hib, DTaP and Hep B<sup>12</sup>. There is no reduction in efficacy when simultaneously administered with OPV<sup>13</sup>. The first dose of Rotarix<sup>TM</sup> is administered between 6-12 weeks of age and interval between two doses is at least 4 weeks. The two dose schedule should be completed by 16 weeks and no later than 24 weeks of age. It is available as lyophilised vaccine to be reconstituted with liquid diluent prior to administration. The Rotateq<sup>TM</sup> is given as three oral doses at 2, 4 and 6 months of age with the 1<sup>st</sup> dose between 6-12 weeks and interval between doses being 4-8 weeks. Vaccination should not be initiated after 12 weeks of age. It is available as a liquid preparation mixed with buffer with no need for prior reconstitution.

### *Human Papillomavirus Vaccine*

Every year 500,000 new cases of cervical cancer are detected and 350,000 succumb annually to this disease<sup>14</sup>. It is well documented

that HPV is the causative agent of cervical cancer with types 16 and 18 accounting for 70% of the cases of invasive cervical cancer globally<sup>14</sup>. These serotypes have also been implicated in anal, vulvar, vaginal, penile and oropharyngeal cancers<sup>15</sup>. Serotypes 6 and 11 cause anogenital warts<sup>11</sup>. Cervical cancer is an important cause of death in Indian women with 132,000 new cases annually with 74,000 deaths<sup>16</sup>. Until recently, cytology based screening programmes (using pap smears) were the main tools to prevent cervical cancers which are capable of detecting upto 80% of cervical cancers in developed countries. However, these are difficult to implement in developing countries with consequent higher mortality. HPV vaccines address a critical public health need and will be an important element of a cervical cancer control strategy.

As of January 2008 two HPV vaccines have been licensed for use in many countries. One is a quadrivalent vaccine containing serotypes 6, 11, 16 and 18 while the second is a bivalent vaccine with serotypes 16 and 18. The vaccines are sterile liquid suspensions prepared from highly purified virus like particles (VLPs) of the recombinant major capsid (L1) protein. The L1 proteins are produced by separate fragmentation in recombinant *Saccharomyces cerviciae* and self assembled in VLPs. The VLPs for each type are purified and adsorbed in aluminium containing adjuvants. The vaccine is available in 0.5 ml single dose ready to use syringes. It is indicated in females between 9-26 years for the prevention of cervical cancer, genital warts, cervical adenocarcinoma in situ, cervical intraepithelial neoplasia grades 1,2,3, vulvar intraepithelial neoplasia grades 2,3 and vaginal intraepithelial neoplasia grades 2,3. The vaccine is administered as three separate intramuscular doses at 0, 2 and 6 months.

Published analysis restricted to females who had not been infected with vaccine related HPV types before vaccination have shown that:

- Both vaccines induce high levels of serum antibodies against HPV types 16 and 18.
- The quadrivalent vaccine had an efficacy of more than 96% in preventing high grade precancerous lesions of the cervix, vagina and vulva and genital warts arising from HPV types 6, 11, 16 and 18 in completed phase 3 clinical trials<sup>17</sup>.
- The bivalent vaccine has an efficacy of more than 90% in preventing high grade cervical lesions due to types 16 and 18 in interim results of phase 3 clinical trials and an efficacy of 75% in preventing persistent infection due to types 16 and 18<sup>18</sup>.
- Although the duration of protection is not yet known there is evidence of protection for at least 6 years after vaccination with both vaccines. Studies are evaluating long term efficacy<sup>19</sup>.

Local side effects were pain, swelling and erythema. No serious vaccine related adverse effects were noted. Recent studies have indicated that both vaccines may provide partial protection against other oncogenic HPV types that are genetically related to HPV 16 and 18. Data on efficacy of the vaccine in preventing diseases in males are not yet available.

In conclusion both the HPV vaccines are safe and efficacious and should be given to all females in the prescribed age group prior to sexual debut.

### *Inactivated Polio Vaccine*

Polio cases have decreased by over 99% since 1988, from an estimated 350,000 cases then to 1997 cases in 2007. This is result of the global effort to eradicate polio. Persistent pockets of polio transmission in northern India, northern Nigeria and the border between Pakistan and Afghanistan are the current focus of the polio eradication initiative. However, in an increasing number of polio free countries the risk of vaccine associated paralytic poliomyelitis (VAPP) is greater than the risk with importation or laboratory handling

of wild polio virus. Some of these countries have introduced IPV as a safe and effective alternative for routine immunisation by using one of the two approaches: replacement of OPV by IPV and introduction of a sequential IPV/OPV schedule.

All currently available IPV are enhanced potency vaccines (eIPV) that contain 40, 8 and 32 D units of types 1, 2 and 3 respectively. It is highly immunogenic and seroconversion rates of 95% and 92% are achieved when two doses are given starting at 2 months of age, at 2 months interval and when 3 doses are given starting at 6 weeks of age and given at 4 weeks interval respectively<sup>20</sup>. It can be given in combination with DTwP and HiB without impairing seroconversion and increasing side effects. IPV also has excellent herd effect<sup>21</sup>. It is a very safe vaccine and is being considered for use in India by the government.

### **Hepatitis A Virosomal Vaccine**

At least 1.5 million clinical cases of hepatitis A occur worldwide each year. The morbidity of hepatitis A is greatest in susceptible adults who are at higher risk of hospitalisation and death. Infected children usually are either asymptomatic or have milder disease but are important sources of infection. As living conditions improve, there is an epidemiologic shift from high to transitional endemicity in many countries which results in more people not previously exposed to HAV and therefore lacking natural immunity. Without natural immunity the risk of outbreaks is increased adding to health costs and making universal HAV immunisation worthwhile.

The successful propagation of the HAV in human derived cell-line culture in vitro in 1979<sup>22,23</sup> paved the way for development of inactivated vaccine. Similar to other small viruses or subunit antigen, inactivated HAV is poorly immunogenic on their own and need some form of immunostimulation in order to be effective as a vaccine. Adsorption to aluminium salt was the only adjuvant for many years<sup>24</sup>. Although widely accepted as safe and effective, aluminium salt based adjuvants have non-specific actions and cause local side effects such as pain and swelling. A new approach has been the development of an aluminium free virosome adjuvanted HAV vaccine. Both types of vaccine are highly immunogenic<sup>25</sup>. Virosomal vaccines have side effects and elicit both cell-mediated and humoral immune responses<sup>26</sup>. In a comparative trial the virosomal vaccine, the aluminium adsorbed formulation and the soluble HAV antigen (no adjuvant), all administered intramuscularly, were assessed for immunogenicity and tolerability. All recipients (100%) of the virosomal vaccine were seroprotected after 14 days compared with 71% of those receiving the aluminium adsorbed antigen and 32% of those receiving the soluble antigen. HAV antibody titres were higher in the virosome group and the virosomal vaccine was better tolerated than the other two formulations.

The virosomal vaccine is available in 0.5ml, single-dose, pre-filled syringes for intramuscular use to be given in two doses 6 months apart. It induces protective antibody level within 10 days of primary vaccination<sup>27</sup> and provides seroprotection for upto 20 years<sup>28</sup>. The two types of vaccine are interchangeable<sup>29</sup>. It can be administered with other vaccines and prophylactic medicines<sup>30</sup>.

### **Conjugated Pneumococcal Vaccine**

*Streptococcus pneumoniae* causes significant morbidity in children less than 2 years of age. It causes over 15-50% of community acquired pneumonia, 30-50% of acute suppurative otitis media (ASOM), significant proportion of bacteremia and meningitis and 30% of mortality due to pneumonia worldwide<sup>31-33</sup>. Studies in patients with

invasive pneumococcal disease (IPD) indicate that serotypes 6, 1, 19, 14, 4, 5, 45, 12, 7, 23 are the most prevalent serotypes with 1 and 5 accounting for 30% IPD<sup>34</sup>.

The earlier *pneumococcal 23 valent*, unconjugated polysaccharide vaccine was a T-cell independent vaccine. It was poorly immunogenic in less than 2 years of age, had low immune memory, did not reduce nasopharyngeal carriage and did not induce herd immunity. It has at most 70% efficacy in high risk population against IPD and does not protect against non-bacteremic pneumonia and ASOM. Not more than 2 lifetime doses are recommended as subsequent doses cause hyporesponsiveness<sup>35</sup>. The *heptavalent pneumococcal conjugated vaccine* (PCV) counters the problems of low immunogenicity of the polysaccharide vaccine in children less than 2 years of age<sup>33</sup>. It contains serotypes 4, 6B, 9V, 14, 18C, 19F and 23 attached to a protein carrier. It covers 85% serotypes causing IPD. Trials show reduction in IPD by 95% and that of X-ray proven pneumonia by 30% in those vaccinated<sup>36</sup>. Efficacy in ASOM is 8%<sup>32</sup>. It has significant herd effect by reduction in nasopharyngeal carriage<sup>37</sup>. There are concerns about serotype replacement with non-vaccine serotypes increasing their share of disease burden with increase in vaccination coverage<sup>38</sup>.

Primary vaccination schedule consists of 3 doses at 2, 4 and 6 months with a booster at 12-15 months.

### **Conjugated Typhoid Vaccine**

Typhoid fever has been reported from all parts of the globe with 16 million cases being reported annually with 600,000 deaths<sup>39</sup>. Until recently typhoid was considered a disease of children above 3-5 years since it presented with atypical clinical features in infants and young children which made it difficult to diagnose. However, a number of studies have shown a high incidence of the disease in less than 2 years ranging from 27-35%<sup>40,41</sup>.

The currently available *Vi polysaccharide vaccine*, though safe and efficacious, is not immunogenic in less than 2 years of age. Hence this age group remains largely unprotected. Moreover, revaccination is required every 2-3 years. The newly introduced conjugated *Vi polysaccharide typhoid vaccine* can be used in infants from 3 months of age with upto 90% protection with longer lasting protection due to immune memory<sup>42,43</sup>. The vaccine is available as a single-dose 0.5ml glass vial for intramuscular use. One dose contains 5 $\mu$ g Vi polysaccharide of *S. Typhi* conjugated to 5 $\mu$ g of tetanus toxoid protein in isotonic saline.

### **Japanese Encephalitis Vaccine**

Japanese encephalitis (JE), a mosquito-borne arboviral infection, is the leading cause of viral encephalitis in Asia<sup>44-46</sup>. Approximately 50,000 sporadic and epidemic cases of JE are reported annually from the People's Republic of China (PRC), Korea, Japan, Southeast Asia, the Indian subcontinent, and parts of Oceania. Infection leads to overt severe encephalitis in only 1 of 20 to 1,000 cases with a fatal outcome in 25% of cases and residual neuropsychiatric sequelae in 30% of cases<sup>44, 47</sup>. In areas where JE is endemic, annual incidence ranges from 1 to 10 per 10,000<sup>44</sup>. Children less than 15 years of age are principally affected. Seroprevalence studies indicate nearly universal exposure by adulthood. In developed countries of Asia and in areas where children are protected by immunization, a secondary increase in JE incidence has been observed in the elderly<sup>49</sup>.

An *inactivated mouse brain derived vaccine* has been mainly used for protection against Japanese encephalitis among travellers and residents of endemic areas. The vaccine has an overall efficacy of

91%<sup>48</sup> with a moderate frequency of local (20%) and mild systemic (10%) side effects<sup>48, 50, 51</sup>. The neural tissue substrate of the vaccine has raised concerns about the possibility of vaccine-related neurologic side effects<sup>(52)</sup>. Protective levels of neutralizing antibody persist for at least 2 years in vaccinees who have completed a three-dose primary series. The full duration of protection is unknown, therefore, definitive recommendations cannot be given on the timing of booster doses. Booster doses of 1.0 mL (0.5 mL for children less than 3 years of age) may be administered after 2 years.

A new Japanese Encephalitis vaccine is undergoing phase III clinical trial. It is manufactured using the Chimerivax technology and as per the trial results it met and exceeded its immunogenicity end point of the trial. The vaccine at present is undergoing pediatric trials in Asia as children are the main target of the disease in endemic regions.

## VACCINES UNDER DEVELOPMENT

1. Malaria Vaccine
2. HIV Vaccine
3. Dengue vaccine

## FUTURE TRENDS IN VACCINOLOGY

1. Development of DNA vaccines
2. Development of therapeutic vaccines.
3. Newer vaccines against non-infectious diseases

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