## Supplemental Table 1: Target diseases in developing countries and/or developed countries

<table>
<thead>
<tr>
<th>Target diseases</th>
<th>Organism responsible for the disease</th>
<th>Main mode of transmission</th>
<th>Mode of action/health consequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diphteria</td>
<td><em>Corynebacterium diphtheriae</em></td>
<td>respiratory droplets</td>
<td>Toxin that destroys body tissues and organs</td>
</tr>
<tr>
<td>Pertussis</td>
<td><em>Bordetella pertussis</em></td>
<td>respiratory droplets</td>
<td>Disease of the respiratory tract</td>
</tr>
<tr>
<td>Tetanus</td>
<td><em>Clostridium tetani</em></td>
<td>contaminated wounds</td>
<td>Potent neurotoxin produced by bacteria in dead tissues</td>
</tr>
<tr>
<td>Haemophilus influenzae type b</td>
<td><em>Haemophilus influenzae</em></td>
<td>respiratory droplets</td>
<td>Can cause pneumonia and meningitis</td>
</tr>
<tr>
<td>Pneumococcal disease</td>
<td><em>Streptococcus pneumoniae</em></td>
<td>respiratory droplets</td>
<td>More than 90 known serotypes; can cause pneumonia and meningitis</td>
</tr>
<tr>
<td>Meningococcal disease</td>
<td><em>Neisseria meningitidis</em></td>
<td>respiratory droplets</td>
<td>Meningococci groups B and C most common in industrialised countries; group A most common in sub-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Saharan Africa; Can cause meningitis or septicaemic illness (high fatality rates).</td>
</tr>
<tr>
<td>Poliomyelitis</td>
<td><em>Poliovirus</em></td>
<td>human-to-human contacts</td>
<td>90% of infected individuals never show any symptoms; when the virus enters the bloodstream it may</td>
</tr>
<tr>
<td></td>
<td>type 1, 2, 3</td>
<td></td>
<td>invade the central nervous system and damage motor neurons, causing paralysis and muscle weakness.</td>
</tr>
<tr>
<td>Measles</td>
<td><em>Paramyxovirus</em></td>
<td>respiratory droplets</td>
<td>Highly infectious disease killing more children than any other vaccine preventable disease.</td>
</tr>
<tr>
<td>Mumps</td>
<td><em>Paramyxovirus</em></td>
<td>respiratory droplets</td>
<td>Primarily affects the salivary glands. Mostly a mild childhood disease, but more severe complications</td>
</tr>
<tr>
<td>Rubella</td>
<td><em>Varicella zoster virus</em></td>
<td>respiratory droplets</td>
<td>Symptoms include rash and fever. Maternal infection in pregnancy may result in fetal loss or congenital</td>
</tr>
<tr>
<td>Hepatitis A</td>
<td><em>Hepatitis A virus</em></td>
<td>fecal-oral route</td>
<td>Acute disease of the liver</td>
</tr>
<tr>
<td>Hepatitis B</td>
<td><em>Hepatitis B virus</em></td>
<td>blood or body fluids</td>
<td>Infection of the liver; acute infections can be severe and may lead to death.</td>
</tr>
<tr>
<td>Rotavirus infection</td>
<td><em>Rotavirus</em></td>
<td>fecal-oral route</td>
<td>Most common cause of severe diarrhea in infants and young children; Seven types of virus species,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>rotavirus A being responsible for more than 90% of infections in humans.</td>
</tr>
<tr>
<td>Influenza</td>
<td><em>Influenza virus</em></td>
<td>respiratory droplets</td>
<td>Can cause mild to severe illness, and sometimes can lead to death.</td>
</tr>
<tr>
<td>varicella</td>
<td><em>Varicella zoster virus</em></td>
<td>human-to-human</td>
<td>Causes fever and itchy rash. The virus remains dormant in the central nervous system of the infected</td>
</tr>
<tr>
<td></td>
<td></td>
<td>contact or droplet spread</td>
<td>individual, and can be reactivated later in life, causing a more dangerous disease known as shingles</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td><em>Mycobacterium tuberculosis</em></td>
<td>respiratory droplets</td>
<td>Usually attacks the lungs (pulmonary tuberculosis), but can also affect other parts of the body,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>including bones and brain. Symptoms may include general weakness, weight loss, fever, and persistent</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>cough and chest pain in pulmonary tuberculosis; may lead to death if untreated.</td>
</tr>
<tr>
<td>Yellow fever</td>
<td><em>Yellow fever virus</em></td>
<td>mosquitoes</td>
<td>Endemic disease in parts of Africa and South America. General symptoms include fever, chills,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>headache, general muscle pain and vomiting, with possible complications such as convulsions, coma</td>
</tr>
</tbody>
</table>
### Supplemental Table 2: Routine immunization schedules for children in the USA and UK

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Birth</th>
<th>1 mo</th>
<th>2 mo</th>
<th>3 mo</th>
<th>4 mo</th>
<th>6 mo</th>
<th>12 mo</th>
<th>13 mo</th>
<th>15 mo</th>
<th>18 mo</th>
<th>19-23 mo</th>
<th>2 y</th>
<th>3 y</th>
<th>4 y</th>
<th>5 y</th>
<th>6 y</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>USA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatitis B (HepB)</td>
<td>HepB-1</td>
<td>HepB-2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HepB-3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rotavirus (Rota)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Rota-1</td>
<td>Rota-2</td>
<td>Rota-3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diphtheria, Tetanus, acellular Pertussis (DTaP)</td>
<td>DTaP-1</td>
<td>DTaP-2</td>
<td>DTaP-3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Haemophilus influenzae</em> type b (Hib)</td>
<td>Hib-1</td>
<td>Hib-2</td>
<td>Hib-3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pneumococcal Conjugate Vaccine (PCV)</td>
<td>PCV-1</td>
<td>PCV-2</td>
<td>PCV-3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inactivated poliovirus (IPV)</td>
<td>IPV-1</td>
<td>IPV-2</td>
<td>IPV-3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Influenza</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Measles, Mumps, Rubella (MMR)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Varicella</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatitis A (HepA)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>UK</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diphtheria, Tetanus, acellular Pertussis (DTaP)</td>
<td>DTaP/ IPV/Hib-1</td>
<td>DTaP/IPV/Hib-2</td>
<td>DTaP/IPV/Hib-3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inactivated poliovirus (IPV)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Haemophilus influenzae</em> type b (Hib)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meningococcal type C vaccine (MenC)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pneumococcal Conjugate Vaccine (PCV)</td>
<td>PCV-1</td>
<td>PCV-2</td>
<td>PCV-3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Measles, Mumps, Rubella (MMR)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 A fourth dose of vaccine is administered, depending on the manufacturer
Supplemental Table 3: Routine immunization schedule for children in developing countries, as recommended by WHO

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Birth</th>
<th>6 wk</th>
<th>10 wk</th>
<th>14 wk</th>
<th>9 mo</th>
<th>&gt;9 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCG</td>
<td>BCG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral Polio Vaccine</td>
<td>(OPV-0)</td>
<td>OPV-1</td>
<td>OPV-2</td>
<td>OPV-3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diphtheria, Pertussis, Tetanus</td>
<td>DPT-1</td>
<td>DPT-2</td>
<td>DPT-3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>HepB-1</td>
<td>HepB-2</td>
<td>HepB-3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scheme A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scheme B</td>
<td>HepB-1</td>
<td>HepB-2</td>
<td>HepB-3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haemophilus influenzae type b</td>
<td>Hib-1</td>
<td>Hib-2</td>
<td>Hib-3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Measles</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Measles)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 In polio-endemic countries
2 Sheme A: In countries where perinatal transmission of HBV is frequent; Scheme B: where perinatal transmission is less frequent
3 A second opportunity to receive a dose of measles vaccine should be provide to all children.
Supplemental file 1: Information on vaccines

The basic information on vaccines and immune responses was extracted from several textbooks and reviews (1-5).

Classification of vaccines

There are four types of traditional vaccines:

Live attenuated vaccines

These vaccines contain live micro-organisms that have been altered to reduce their pathogenic potential. They still retain some of the antigens of the virulent form and these antigens generate protective immunity. Immunity conferred by these vaccines is generally long-lasting (>10 y), but may wane in the absence of repeated exposure to the wild organism (e.g. measles). However, they also carry some risk because the microorganisms injected might mutate back to the virulent form. Live attenuated vaccines are not recommended for use in recipients with weakened immunity, as they may themselves produce disease. Examples of these vaccines include yellow fever, measles, rubella, and mumps.

An alternative way of making a live vaccine is to use a closely-related but less dangerous organism to produce an immune response, such as Jenner did with his use of the relatively mild cowpox virus to protect against the similar but often deadly smallpox virus. Another example is the Bacillus Calmette-Guerin (BCG) which is a weakened version of the bacterium that causes tuberculosis in cows.

Inactivated (killed) vaccines

These are previously virulent micro-organisms that have been killed with chemicals or heat to render them harmless. They cannot cause an infection, but they do stimulate a protective immune response. They may require an adjuvant and/or a booster vaccination. Examples are vaccines against flu, hepatitis A and cholera.

Toxoid vaccines

Some vaccines are made from the protein toxin liberated by the bacterium. The toxin is chemically modified to decrease its harmful effects. After such treatment the toxin is called a toxoid. Examples of toxoids are the diphtheria and tetanus vaccines. Vaccines made from toxoids are usually administered with an adjuvant to improve their immunogenicity and require booster vaccinations (<10 y). In return, they can be recommended as fully safe for the vaccinees.

Subunit vaccines

Rather than introducing the whole micro-organism (killed or attenuated), only a fragment of it – for example the capsule, the flagella, or part of the protein cell wall - can be used to generate an immune response. This technique requires identifying the peptides encompassing the major antigenic sites of viral antigens, from which highly purified subunit vaccines can be produced. Increasing purification may lead to loss of immunogenicity, so a coupling with an adjuvant and booster doses of the vaccines may be required to ensure their continued effectiveness. These types of vaccines cannot cause the disease and are therefore considered to be safe for use in immuno-compromized
patients. A characteristic example is the vaccine against Hepatitis B which is composed of only the surface proteins of the virus.

A number of innovative vaccines are also in development or in use:

**Polysaccharide vaccines**

Immunity to encapsulated bacteria is mainly generated by antibodies directed against the polysaccharide capsule. Vaccines have been developed from the capsular polysaccharide against organisms such as pneumococcus, *Neisseria meningitidis* and *Salmonella typhi*. These vaccines induce a T cell-independent response which is not boostable and does not generate immunological memory. Children under 2 y of age do not respond well to such antigens, although this varies with the antigen and some types of *N. meningitides* and pneumococcus are immunogenic in young infants. Poor immune responses to a polysaccharide vaccine to Hib led to the development of conjugate vaccines.

**Conjugate vaccines**

By linking polysaccharides to immunogenic proteins, such as toxins, the immune system can be led to recognise the polysaccharide as if it was a protein antigen. This results in a vaccine that produces a T cell dependent, boostable immune response that is effective, even in very young infants. This approach was used to develop the Hib vaccines, and recent vaccines against pneumococcus and *N. meningitidis*.

**Recombinant vaccines**

Recombinant DNA technology has allowed the production of hybrid virus vaccine, where the gene coding for the antigen is inserted into a vector, usually a virus that has a very low virulence. The vector expressing the antigen may be used as the vaccine, or the antigen may be purified and injected as a subunit vaccine. The only recombinant vaccine currently in use in humans is the Hepatitis B virus vaccine, which is a recombinant subunit vaccine. Hepatitis B surface antigen is produced from a gene inserted into yeast cells and purified for injection as a subunit vaccine.

**DNA vaccines**

DNA vaccines are the newest vaccines and are still experimental, although encouraging results on their effectiveness and safety have been reported. As for the recombinant vaccine, genes coding for the foreign antigens are located and cloned. The difference is that the DNA is then directly injected into the muscle. Once injected the DNA is taken up by the host cells, which then start expressing the foreign antigen. The protein serves as an antigen that stimulates an immune response and protective immunological memory. In contrast to conventional vaccines, DNA vaccines elicit cell-mediated as well as antibody-mediated immune responses. In theory these vaccines are extremely safe and devoid of side-effects since the foreign antigens are directly produced by the host. In addition DNA is relatively inexpensive and easy to produce and store. However, the enthusiasm for DNA vaccination in humans is hampered by the fact that delivery of the DNA to cells is still not optimal. Another concern is the possibility that the vaccine’s DNA would be integrated into host chromosomes and would turn on oncogenes or turn off tumor suppressor genes. Furthermore, extended immunostimulation by the foreign antigen may cause chronic inflammation or autoantibody production.
Vaccines with adjuvant

Adjuvants or immunopotentiators are defined as components added to vaccine formulations that enhance the immune response to vaccine antigens in vivo. Advantages of vaccine adjuvants include the enhancement of the immunogenicity of antigens, the modification of the nature of immune response, the reduction of the antigen amount needed for a successful immunization, the reduction of booster dose needed, or the enhancement of immune response in individuals with weakened immune systems (for example, children, elderly or immuno-compromized adults). Adjuvants can act in different ways in order to elicit a protective immune response against an antigen, but the different mechanisms involved are only partially understood. Nevertheless, adjuvants have been functionally classified into two major categories based on their dominant mechanism of action: i) immune potentiators which activate the innate immune system and ii) delivery systems which ensure that vaccine antigens are delivered to the right place at the right time. Immune potentiators can activate innate immunity either directly (for example cytokines) or through pattern-recognition receptors which are expressed on a wide variety of immune cells and lead to their activation when engaged with an antigen. In contrast, delivery systems are presumed to be immunologically inert since no receptors have been identified so far. They facilitate residence time, place and dose of the antigen after injection. Examples include aluminium salt and oil-in-water-based emulsions.

Many adjuvants have been developed in the past, but were never licensed for use in routine vaccination because of safety concerns, i.e. acute toxicity and potential delayed side-effects. Nowadays there is an increasing development of adjuvants, and the current attitude is to use new and safer adjuvants to strengthen weak immunogens. As an example the new malaria vaccine RTSS contains no fewer than four different principles of adjuvanticity in the AS02 adjuvant used.

Immune responses to vaccines

Vaccines protect populations against infectious agents by inducing active immunity and providing immunological memory. This memory primes the immune system to recognize and respond rapidly to a later exposure to natural infection and thus prevent the disease. The response to vaccine antigens is a complex and regulated series of events involving several cell types and signalling pathways. The process can be broadly divided into antibody-mediated (B lymphocyte) and cell-mediated (T lymphocyte) components, these two branches being closely inter-related.

Antibody-mediated or humoral responses

B lymphocytes differentiate in the bone marrow. Immunoglobulin (Ig) receptors on B lymphocytes recognize and interact with antigens triggering coreceptor-mediated signalling pathways that lead to differentiation into plasma cells and replication to memory B cells. In addition, the Ig-antigen complex is endocytosed and then processed within the cell for antigen presentation to T cells. Concomitantly, activated antigen-specific T cells provide help to further promote the B cell response. Plasma cells produce and secrete different subclasses of antibodies (IgM, IgG, IgA, IgD and IgE in mammals), each of which mediates a characteristic biological response following antigen binding. Antibodies bind antigens through noncovalent bonds. The strength of interactions between a single antigen-binding site on an antibody and a single epitope of an antigen is called the affinity of the antibody for this epitope. Low-affinity antibodies bind antigen
weakly and tend to dissociate quickly, whereas high-affinity antibodies bind antigen more firmly and remain bound longer. When antibodies contain multiple binding-sites and bind complex antigens at different sites, the strength of these multiple interactions is called avidity, which is a better measure of the real strength of the antibody-antigen interaction.

Once secreted, the antibodies circulate in the body and provide immunity against antigens located in the extra-cellular space in various ways: by neutralizing toxins; by blocking adhesion and cell entry by organisms; by neutralizing and preventing viral replication; by opsonization where the pathogen is marked for destruction by a phagocyte. Following these steps, some of the B cells will die and some will further differentiate to become memory B cells, which circulate around the body. When these cells encounter antigens again in lymphoid tissues, the cycle of differentiation may begin again, resulting in the production of more antibodies. Thus, the purpose of a boosting dose of antigen through vaccination is to induce the differentiation of existing B memory cells and to enhance the number of antigen-specific B cells.

Recent research suggests that B cells might be actively involved in the regulation of the immune response (reviewed in (6,7)). New subsets of B cells have been delineated, and specific B regulatory cells have been identified in mice. These cells have the capacity to restrain immune responses, thus playing a major role in preventing destructive autoimmunity. Whether a similar B regulatory cell population exists in humans is not clear yet. A CD20+ CD25+ B cell subset has recently been identified in humans, which could play a role in immune regulation through the secretion of interleukin (IL) 10 among other factors.

**Cell-mediated responses**

Cell-mediated responses are controlled by T lymphocytes which differentiate in the thymus. Unlike B cells, T cells circulate directly to the site of antigen exposure, i.e. the lymphnode which drains the tissue where the infection or vaccine application happened. There, T cells are primed and activated to become effector cells against the specific antigens. Subsequently, activated T cells migrate directly to the site of infection/vaccination. T cells only recognize, via T cell receptors, fragments of antigens that have been processed and combined with major histocompatibility complex (MHC) molecules on the surfaces of antigen presenting cells (APC). The two main subpopulations of T cells - T helper (Th) or CD4+ cells, and T cytotoxic (Tc) or CD8+ cells - recognize antigenic peptides associated with class II and class I MHC molecules respectively. Co-stimulatory molecules (e.g. various cytokines or stimulator expressed by the APC), for which T cells also have receptors, are also required for activation of T cells. Th cells play a critical role in immune responses, since once activated, they secrete various cytokines that will induce the activation of B cells (thus the production of antibodies), Tc cells, macrophages and other cells involved in the immune response. Depending on the cytokine and transcription factor signals they receive during their activation, Th cells differentiate into Th1, Th2, Th17 and induced regulatory T cells (Tregs), and possibly into other yet unknown subsets. Each of these subsets have specific cytokine-production profiles and effector functions, thus inducing different immune response pathways. Th1 cells are thought to be critical to immunity to intracellular pathogens and to stimulate cell-mediated immunity, whereas Th2 cells are critical in the fight against extracellular pathogens and in stimulating humoral immunity. A Th1/Th2
imbalance can cause host tissue damage, for example organ-specific autoimmune diseases from uncontrolled Th1-type responses, or allergies and asthma from uncontrolled Th2-type responses. However, the Th1/Th2 balance concept is still poorly understood, especially in humans, and probably too simplistic. Th17 cells, which have been recognized much more recently, are believed to play a critical role in host defense against specific extracellular bacteria and fungi. They may also be involved in the induction of tissue inflammation and autoimmune diseases. The fourth Th cell subset, induced Tregs, has the ability to regulate the immune responses by suppressing the activation of T cells. Those Tregs include two distinct subtypes of CD4+ T cells: T regulatory 1 (Tr1) cells, which exert their suppressive action through secretion of IL-10, and T helper 3 (Th3) cells which act through the transforming growth factor β (TGF-β) (8,9). Natural (non-induced) regulatory T cells, which express the cell-surface marker CD25 and the transcriptional repressor FOXP3 (FoxP3+ Treg cells) also exist. These cells mature and migrate from the thymus to peripheral tissues, where they suppress the immune system in a cell-contact-dependent manner, in order to prevent overreactive responses and to regulate inflammation caused by T cells and their downstream effector cells. In particular, the type of cells that are known to be suppressed by FoxP3+ Tregs include CD4+ and CD8+ T cells, natural killer cells, dendritic cells, monocytes macrophages or B cells (10).

Whilst Th cells have a strong role in the regulation of immune responses, in contrast the major role of Tc cells is to recognize and lyse infected target cells. After recognition of an antigen-class I MHC complex and activation by Th cell-derived cytokines, such as IL-2, Tc cells proliferate and differentiate into effector cells called cytotoxic T lymphocytes. After the rapid expansion of responding T cells during an antigenic challenge, and once the danger is cleared, most effector cells will die. Memory T cells (both cytotoxic and helper) will survive and circulate freely around the body. Those cells may then be stimulated very easily and quickly, resulting in a quick and stronger response in case of a new antigenic challenge (booster dose of a vaccine or infection).

Immune responses induced by different vaccines

Different vaccines induce different immune responses (Table 3). Live attenuated vaccines induce the full range of functions (i.e. B cells, CD8+ T cells and CD4+ T cells) and drive primarily a Th1-type response, whereas killed vaccines and protein antigens induce B cells, CD4+ T cells and drive primarily a Th2-type response. Polysaccharides only induce B cell responses unless conjugated with a carrier protein, in which case they act like protein antigens. Like live vaccines, DNA vaccines induce the full range of immune response types, but in addition, they can be manipulated to turn the T cell help towards Th1 or Th2 response. Vaccine adjuvants may also modulate the immune response that develops. As an example, aluminium based adjuvants are excellent at promoting humoral and Th2-type responses, but poor at generating adequate cell-mediated immunity and long term T cell memory (11). The recent advances in basic immunology, in particular the identification and characterization of new lineages of cells such as Th17 and Treg cells, have offered new possibilities in adjuvant and vaccine development (12,13). Research investigating the generation and functional role of these
key regulatory cells and associated cytokines in vaccine immunogenicity in humans is required.
Supplemental Literature cited


Supplemental file 2: Factors affecting vaccine responses

While most currently produced and used vaccines elicit a protective response in most recipients, several factors can influence the nature and strength of the response in different individuals.

Age

Age at vaccination influences the immune response to several vaccines, with clear differences between neonatal and adult immune responses both in mice and humans with respect to both humoral and cell-mediated immunity.

Maternal acquired antibodies

The persistence of placentally-transferred maternal antibodies has been reported to be a factor limiting appropriate vaccine responses in young children, although research related to this topic has yielded contradictory findings (1,2). Inhibition of vaccine response by maternal antibodies has been observed with several vaccines, including live measles and oral poliomyelitis vaccines (3,4), and non-live vaccines such as pertussis, diphtheria and tetanus toxoids (5,6), Hib (7,8) and hepatitis A vaccines (9,10). Other studies have reported no effect of maternal antibodies on the immune response to the same vaccines (11-14). A crucial determinant in this inhibition seems to be the level of maternal antibodies at the time of immunization. Thus the heterogeneity of maternal antibodies in an infant population would explain the contradictions observed in clinical studies performed in different populations or at different ages. Circulating maternal antibodies, achieved through the placenta mainly in the third trimester of pregnancy or through breastfeeding, may impair the vaccine response possibly by neutralising vaccine antigens, thus preventing the infant’s B cells from binding these antigens. The existence of a suppressor mechanism which down-regulates the antibody formation when sufficient antibodies are present has also been hypothesised. However, it has been shown that presence of maternal antibodies did not affect induction of infant T cell responses to live replicating measles vaccine (15), suggesting that maternal antibodies may leave T cell responses unaffected.

The amount transferred and the persistence of maternal antibodies in children may vary according to several factors, including the vaccination status of the mother. In their review, Leuridan et al. (16) have documented that mothers vaccinated with live attenuated measles vaccine had lower amounts of antibodies and passed on shorter-term protection against measles to their children than mothers who were naturally infected. Genetic and environmental factors are also suspected to have an influence in this transmission (17). In addition, regional variations in the persistence of maternal antibodies in their children have been described. Children in Africa seem to loose the antibodies transmitted by their mothers much faster than children from developed countries, suggesting that this loss may also be influenced by co-factors such as malnutrition or co-infections (18-20). The best way to improve vaccine efficacy and reduce primary vaccine failure is therefore to schedule vaccination long enough after birth to have the minimum interference with maternal antibodies. However, this strategy places unvaccinated infants at higher risk of infections, which are often the most severe at this time of life. An alternative is to increase the dose of vaccine antigen, which increases the B-cell epitopes likely to escape from maternal antibody bindings, and thus remaining
available for infant B cells. This strategy was successful with different vaccines both in animals and human studies (21).

Vaccine responses in childhood and adolescence

In infants, maturation of the immune system continues after birth. Neonates show very poor responses to both polysaccharide and protein vaccines. This immunological immaturity may involve B cells and/or their interaction with antigen-presenting cells, T cells, follicular dendritic cells or other components of the lymphoid environment (22). For this reason, almost all vaccines developed to date require several doses to elicit protection when administered before 6 mo of age. BCG vaccine is one of the exceptions. However, despite an initial weak antibody response, some vaccines may activate neonatal B cells and trigger their differentiation into memory B cells. This mechanism represents important immunological priming which can enhance future responses. For example, it has been demonstrated that the first OPV dose administered to neonates induced no or weak primary responses but a significantly higher increase in antibody levels to the second vaccine dose than in unprimed infants (23).

Age may also be an important factor for later immunization, in particular for maintenance of antibody levels after immunization with certain vaccines. For example, a recent study has observed that serogroup C meningococcus specific antibodies were more persistent 5 y after immunization in children aged 10 y or above than in younger age groups (24).

Sex

Gender may influence the antibody response to some vaccines. A recent review has identified 97 studies reporting sex-difference in humoral response in humans (25). Females have been reported to have greater antibody responses than males following vaccination with hepatitis A, hepatitis B, rubella, and tetanus vaccines. In contrast, males were reported to have greater immune responses to pneumococcal, meningococcal A and C and yellow fever vaccines. Sex-differences in antibody response were also found with influenza, measles, diphtheria and rabies vaccines, but findings were more contrasted, with sometimes better immune responses in females and other times in males. These variations were mainly due to differences in individuals' age between studies or whether it was a primary or boosting vaccination, but considering all the vaccines together there was much more evidence towards a stronger antibody response in females than in males. Other reviews reported considerable evidence showing that females generally have stronger humoral and cellular immune responses than males (26). Female animals from different species have higher serum concentrations of immunoglobulins and show higher and more sustainable production of antibodies after immunization and infections. Therefore females may be globally more resistant to certain infections, such as bacterial infections, and suffer a higher incidence of autoimmune diseases as compared with males (27). Observations that females have a higher number of T helper cells, and a better increase of these cells after vaccination than in males may partly explain the stronger antibody response in females (28,29). The Th1/Th2 balance may also be involved, with women having a predominant Th2 cytokine profile compared to men (30). One hypothesis behind such gender differences may be interactions between sex hormones and immune factors. Oestrogens have been shown to depress T cell mediated and stimulate B cell mediated immune responses, while progesterone and androgens depress
both components of the immune system. Oestrogen also regulates non-specific immune response, in particular the number and function of neutrophils and natural killer cells through anti-inflammatory effects. In contrast progesterone exerts pro-inflammatory effects on neutrophils (31). However, other mechanisms might be involved since sex differences in vaccine responses have also been reported in pre-pubertal and post-menopausal subjects not on hormone replacement therapy.

Prematurity

Premature infants (<37 wk gestational age) are at increased risk of disease from a number of vaccine preventable diseases. This vulnerability is mainly due to the immaturity of their immune system and a deficit in maternal antibodies, such as immunoglobulins G which cross the placenta mainly in the last 4 to 6 wk of gestation. Early protection is therefore desirable in preterm infants; yet, immunization schedule are often delayed in these infants (32) because of doubts about the immunogenicity, efficacy and safety of certain vaccines administered to preterm infants. A number of studies have examined the efficacy and safety of vaccines in preterm infants, showing opposing findings according to the vaccine considered.

**Hib, hepatitis B and influenza vaccines**

The immunogenicity of Hib conjugate vaccines varies widely among studies of premature infants, probably because of variation in the choice of the conjugate protein, inclusion in a combination vaccine or time schedule of the doses. Hib antibody levels appear to be lower in preterm infants compared to term infants after each of the first two doses (33-36). Similar findings were observed after the third dose only when the time schedule used was 2, 3 and 4 mo of age, but not when extended schedules such as 2-4-6 mo or 2-4-12 mo were used (34,37,38).

Immune response to Hep B vaccination may also be weaker in preterm than in full-term infants, and appears to be dependent on the infant’s weight at the time of immunization. One study has observed that Hep B vaccine given at birth was poorly immunogenic in infants with birth weights < 2000 g, and that delaying the administration until the infant reached 2000 g or 60 d of age resulted in a better immunogenicity (39). Subsequent studies later confirmed that almost all preterm infants became protected against hepatitis B after the third dose of the vaccine if they had received the first dose after 30 d of age, regardless of their gestational age and birth weight (40).

No study has examined the immune response to influenza vaccines in preterm infants younger than 6 mo of age. Therefore, the vaccination of this age group is not recommended and their protection may be achieved instead by the immunization of the family. The only data available on older infants have suggested that inactivated influenza vaccine administered at or after 6 mo of age seems to be less immunogenic in preterm than in term infants (41).

**Meningococcal and pneumococcal vaccines**

It has been shown that MenC vaccine was equally safe and immunogenic in both preterm and term infants when administered on 2-3-4 mo or 3-5-11 mo schedules (10), with comparable post-immunization antibody levels and antibody persistence to 12 mo (10,35). However, preterm infants had a lower response than term infants to a booster dose administered at 12 mo of age; this booster dose is essential for long-term protection.
Similar results were observed with the conjugate pneumococcal vaccine. Several studies have shown that preterm infants developed comparable antibody levels to term infants after 3 doses of the 7-valent conjugate pneumococcal vaccine administered with a 2-4-6-12 mo or 3-5-11 mo schedules (43,44). Another UK study observed that although preterm infants had reduced antibody concentrations compared to term infants, both groups had antibody levels well above the protective threshold (45).

**DTP, Polio and BCG vaccines**

Several studies have shown that preterm infants (32-36 wk of gestational age) receiving DTP and polio vaccines at 2, 3 and 4 mo of age had similar immune responses to full-term infants, although antibody levels were significantly lower after the first dose of vaccine (46,47).

Conflicting results on reaction to tuberculin in BCG-vaccinated preterm infants have been reported; hence the optimum time of vaccination in such babies is still under discussion. Some studies have shown that low birth weight and preterm infants had similar, and even more efficient immune response to BCG vaccination compared to control infants, as assessed by tuberculin conversion rate or in vitro lymphocyte proliferation (48-50), while other studies showed that preterm babies had less BCG scaring and tuberculin conversion than full-term babies (51,52).

**Long-term protection**

Only a few studies have assessed the long-term immune response in preterm infants to several vaccines. Kirmani *et al.* (53) have assessed the response to Hib, Hep B, DTP and polio vaccines of 7-y-old children born extremely premature. Children who were born premature had lower antibody levels to these antigens than children born at term, although for diphtheria and tetanus vaccines they both maintained antibody levels in the protective range. Other studies have shown that low birth weight may have long-term impaired immune response to certain vaccines (54,55).

**Season of administration**

Very few studies have looked at the influence of the season of vaccine administration on the immune response to this vaccine. Yet, it is known that nutritional status and infections may affect vaccine responses, and that these factors are strongly patterned by seasonality in some regions of the world. Moore *et al.* (56) specifically examined the effect of the month of vaccine administration on antibody responses, in The Gambia and Pakistan, two countries with distinct patterns of seasonality. In Gambian children significant associations between month of vaccination and antibody response were observed for pneumococcal and rabies vaccines, while no monthly influences were observed in the response to tetanus, diphtheria or hepatitis B vaccines in younger infants. In Pakistani adults, the antibody responses to both rabies and typhoid vaccines were significantly influenced by the month of administration of the vaccines. Although the precise mechanism involved needs to be further elucidated, it was suggested that seasonally-dependent environmental antigens could have an adjuvant effect on the vaccine, thus enhancing the antibody response at specific times of the year. In addition other seasonally dependent factors such as nutritional status, infections or background antigen exposure may have a co-stimulatory effect in these observations. In The Gambia, Deming *et al.* (57) have shown that administration of OPV vaccine during the rainy
season was associated with an increased risk of vaccine failure as assessed by the incidence of poliomyelitis in the children vaccinated.

Other studies have reported seasonal variation in markers of immune function, such as blood leucocytes and lymphocytes subpopulations (28,56), cell-mediated immunity assessed by skin test response to test antigens (58), or the size of the thymus in infants (56). All these findings tend to confirm that seasonality may be an important factor influencing vaccine response.

**Background exposure**

*Subclinical infection*

Natural immunity may be acquired after exposure to the pathogen, but before vaccination against this pathogen. For example, repeated exposure to Hib type b during childhood enables acquisition of natural immunity against the bacteria by production of functional antibodies. This natural immunity may then reduce immune response induced by subsequent Hib vaccination. Moreover concerns have been raised about the possibility that widespread use of conjugate Hib vaccines could reduce circulating Hib bacteria, and thus lead to the progressive waning of natural immunity in unvaccinated groups (59). In contrast, natural subclinical infections occurring after immunization may boost antibodies induced by vaccination and help maintain long-term immunity. A study conducted in Senegal has suggested that subclinical measles infections in vaccinated children resulted in a significant increase in measles antibodies which remained raised for at least 6 mo (60).

**Cross-reacting antigens**

Infections with environmental pathogens which have similar, or at least closely related, epitopes to those of the vaccine component may also interfere with the vaccination response to such component, a phenomenon called cross-reaction. To pursue with the example of Hib, it has been shown that contacts with other bacteria with capsular polysaccharide that are antigenically similar to Hib, e.g. *Escherichia coli*, may induce the production of Hib specific antibodies (61). Similarly, the various mycobacteria (*Mycobacterium avium*, *M. scrofulaceum*, etc.) present in the environment are known to cross-react with the *Mycobacterium bovis* used in the BCG vaccine and *M. tuberculosis* responsible for TB disease. Findings from various studies have shown that a higher exposure to such environmental bacteria was associated with a lower responsiveness to the BCG vaccine, as assessed by delayed type hypersensitivity skin testing and IFN-γ production (62,63). The environmental mycobacteria being very different in different localities, this phenomenon is likely to explain the geographical variation observed in the efficacy of BCG vaccination (64).

Both (sub)clinical infections prior to vaccination and cross-reactivity may lead to a reduction in the vaccine-induced immune response. Possible mechanisms behind this may be that exposure to environmental microorganisms provides partial protection which may restrict the growth or replication of the vaccine component and thus makes the vaccine ineffective. It may also be that the vaccine is unable to confer any additional immunity than that already induced by the natural exposure to the pathogen. On the other hand, background exposure to antigens may be useful as a natural booster following vaccination.
Ethnic and genetic factors

Interindividual variations in immune responses to vaccines can be partly explained by the genetic background of the recipients. The heterogeneity in vaccine-induced immunity, also called “vaccinomics”, is a growing area of research which seeks to understand the influence of immune response gene polymorphism on the heterogeneity of humoral, cell-mediated and even innate immune responses to vaccines at both the individual and population levels. The heterogeneity in vaccine-induced immunity is still not well understood, and as pointed out by recent excellent reviews (65-67), little data are available from genetic studies worldwide, and even less in Africa. Twin studies provide an ideal method for understanding the contribution of genetic factors to variation in the immune response, and despite their limited numbers, they have shown that immunity induced by vaccination is heritable (68-71). The genetic influence on immune response has been further indicated by racial and ethnic differences in vaccine responses, especially with Hib, measles and BCG vaccines (68-70). These types of studies are very complex given the numerous interactions between genetic and nongenetic factors (e.g. household size, nutritional and economic status), and genetic mechanisms underlying ethnic differences are poorly understood.

The immune response to vaccines is complex, and every step in the induction and effector phase of this process is under control of various host genes. Polymorphisms of any of the involved genes may affect the immune process and resultant response. The HLA region, implicated in the process of antigen presentation, is the most polymorphic region known in the human genome, and is therefore highly influential in interindividual immune response differences. Many studies have reported associations between the HLA alleles and the immune response to hepatitis B vaccine (72-74) and to measles vaccine (75,76). Associations between HLA genotype and immune responses have also been reported for other vaccines, including influenza, rubella or BCG vaccines, although studies are sparse (reviewed in (65,67)).

Despite the recent advances in genetic technologies, it is clear that the “vaccinomics” area is still in its infancy. Further research is necessary to identify new candidate genes and increase the understanding of mechanisms underlying vaccine-induced immunity, thus allowing development of new and improved vaccines.

Breastfeeding

Human breast milk contains many components beneficial for the infant’s immune system, including antibodies, leukocytes or antimicrobial factors (55,77,78). It has been suggested that breastfeeding may enhance the infants’ response to vaccination. A long lasting enhancing effect was observed for the IgG2 antibody response to vaccination against *Haemophilus influenzae* type b (79). This result has been confirmed recently and the study expanded to highlight similar effects of breastfeeding on the antibody response to pneumococcal vaccination (80). Exclusive breastfeeding for at least 90 d leads to a higher proportion of infants with vaccine responses at protective levels, compared to children less breastfed. Several studies have shown increased immune response to BCG, poliovirus, diphtheria, tetanus and *haemophilus influenzae* type b vaccines in breastfed versus formula-fed babies (81-83). However, other studies have not found such positive effects (84-86) or even significant adverse effects on rotavirus vaccine seroconversion (53) and oral poliovirus vaccine efficacy/success (27). Such differences in the findings
may be the result of variations in the levels of maternal acquired antibodies against the vaccines used, variations in the composition of mother’s milk or in the type of vaccines tested. Indeed, as suggested by Jackson et al. (78), studies showing no effects or adverse effects of breast-feeding on childhood vaccinations often used live viral vaccines whose immunogenicity may be inhibited by the secretory IgA contained in human milk. This is particularly likely for orally administered vaccines.

Breast milk can also partially protect newborns against certain infections, through the transmission of mother’s antibodies. Although secretory IgA is abundant in human breast milk, other antibodies are not transmitted to the neonates in substantial amount. Secretory IgA found in human milk has been found to be protective against bacterial pili, pneumococci, Hib type b and poliovirus (reviewed in (87)). Partial measles immunity in the first few weeks of life can also be achieved through breast milk, in particular measles-specific IGA (88). However, a study conducted in Nigeria showed that anti-measles IgA dropped below protective cut-off values within 2 wk after birth (89). Specific antibodies found to be elevated in breast milk after maternal immunization also include antibodies against meningococcal and pneumococcal diseases (reviewed in (90)).

Obesity
Although it is not yet an issue in children living in developing countries, there is a growing body of literature from both adults and genetically obese rodents showing that obesity results in impaired immune function. Several studies also suggest that obesity is associated with a poor antibody response to certain vaccines, in particular hepatitis B vaccine (91,92).

Other factors
Many other factors are known or believed to influence immune response to vaccines. These are not the focus of this review and thus not discussed in detail here. Such factors include various parameters related to the vaccine itself (type, doses, administration route, etc.), but also psychological stress, smoking, infectious diseases, UV light exposure and others (93).
Supplemental Literature cited


