Vaccination against Bacterial Infections

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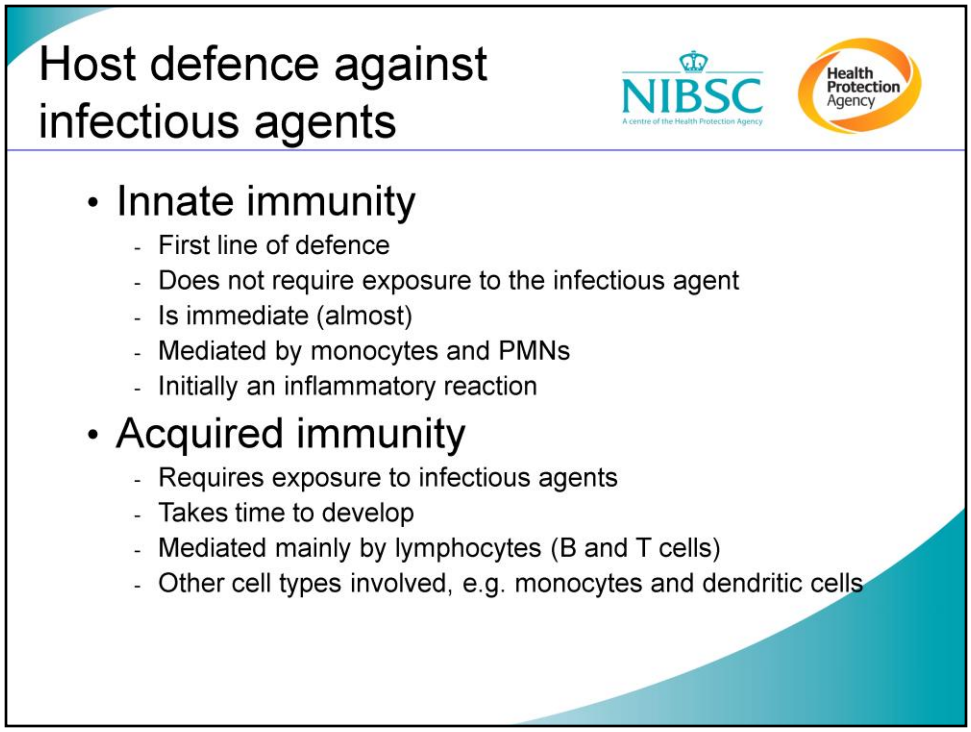
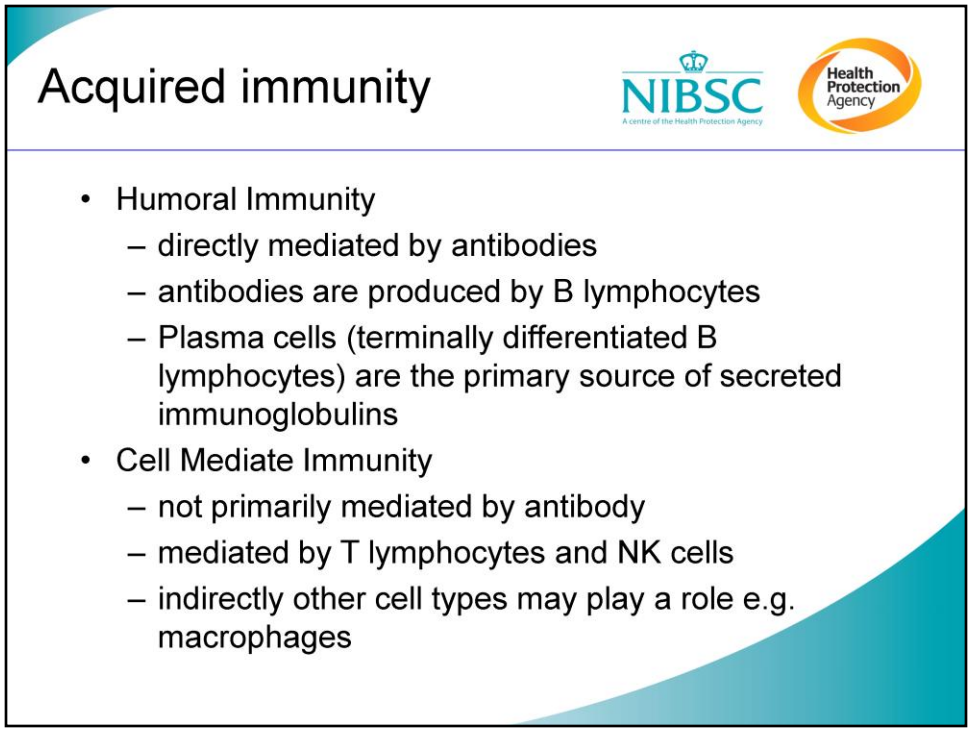
Overview

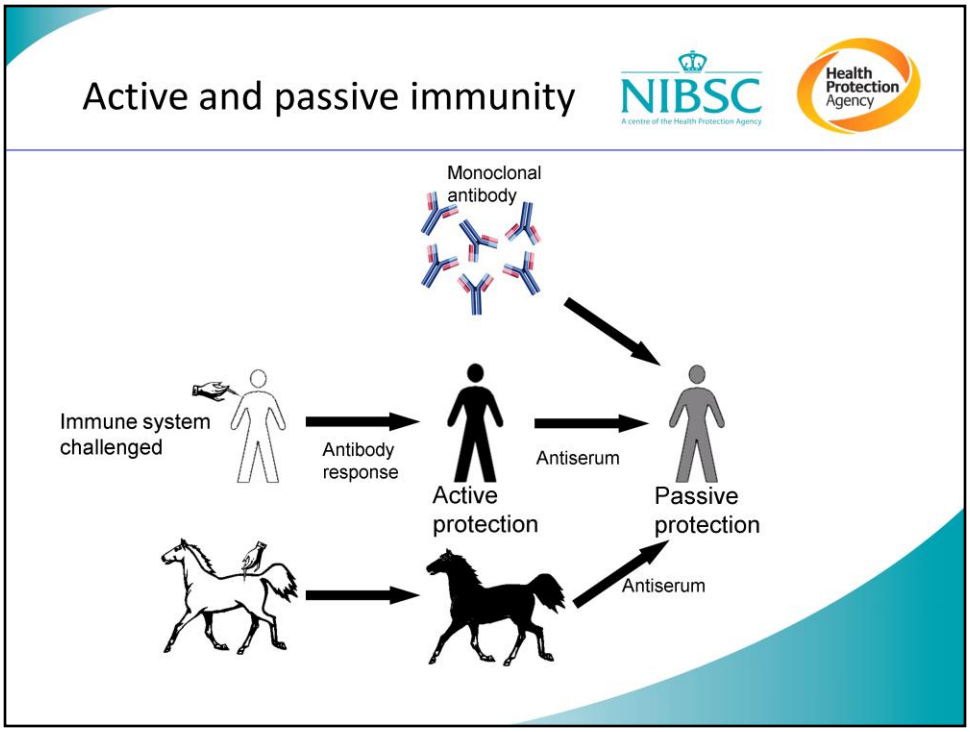
- Background on host defences and how this relates to vaccination
- Different types of vaccines
- What's in a vaccine formulation?
- New vaccines on the horizon



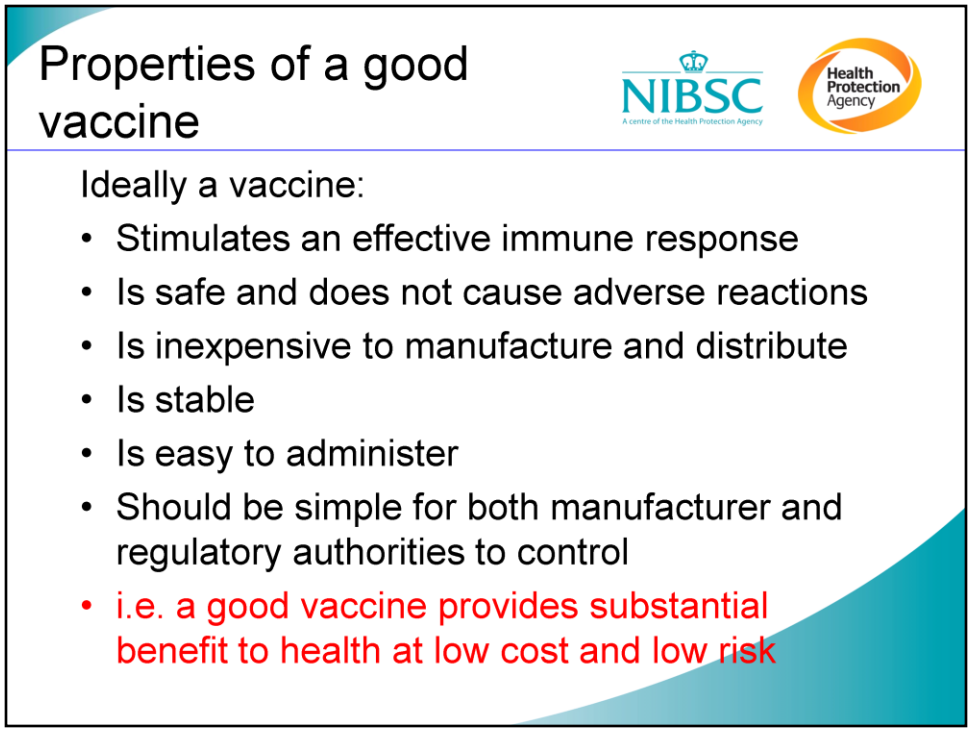
Host immunity can be broadly divided into two types: innate and acquired immunity. Vaccines stimulate both aspects of the immune response but are primarily aimed at eliciting acquired immunity which requires exposure to the infectious agent or its antigens.



Acquired immunity can be divided into two arms: humoral immunity which is mediated by antibodies and cell mediated immunity. It is important that a vaccine stimulates an appropriate immune response. For example, humoral immunity is usually important for preventing septicaemia whereas cell mediate immunity is often important for the prevention of intracellular infections. They are not mutually exclusive with both elements of the immune response playing a role in protection against may diseases.



Active immunity is elicited in the host in response to an antigen. Passive immunity is the acquisition of protection from another immune individual through transfer of antibody or activated T-cells. The purpose of a vaccine is to induce active immunity.



In many respects these are common sense points.

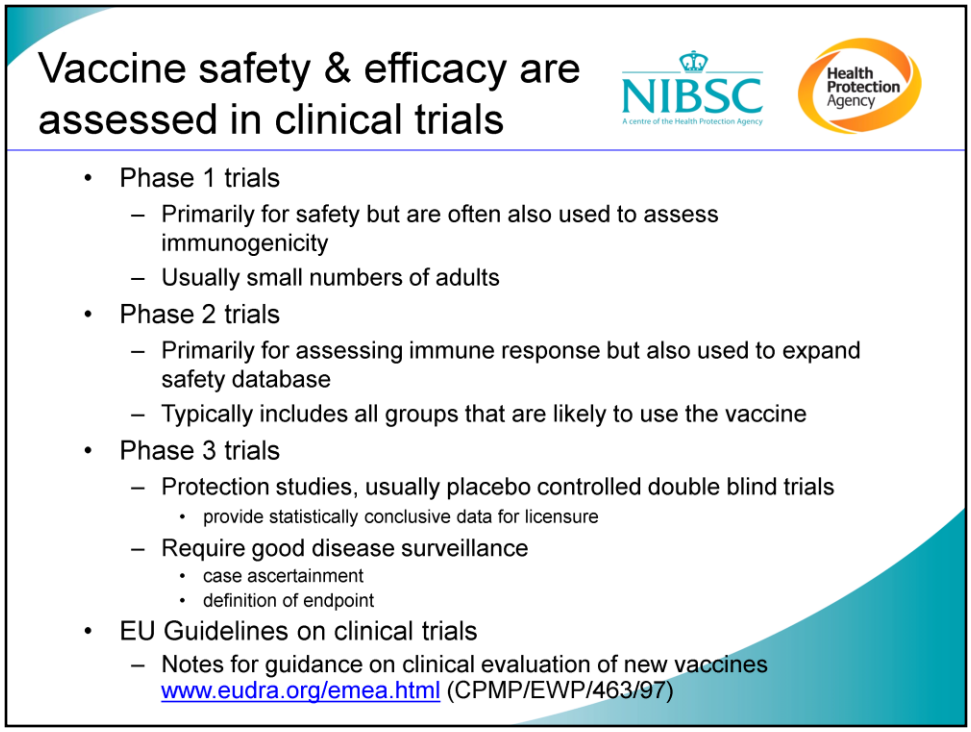
To be effective an immune response should be appropriate to the disease in question.

- It should elicit the correct balance of humoral and cell mediated responses
- The immune response should be directed to the relevant site within the host (e.g. the gut in the case of enteric infections).
- The immune response should be "functional". For example, toxin-neutralising antibodies should be able to bind to toxin and neutralise its activity or if bactericidal antibodies are required the antibody should bind both the bacterium and complement.

An effective vaccine would be expected to elicits this response in all (or at least the majority of) vaccinees, give life-long protection without repeated doses, stimulate a boostable response and offer protection against antigenically diverse strains

Vaccine safety is critical as they are given prophylactically to healthy individuals, who should remain healthy after vaccination.

- A parenterally administered vaccine must be sterile (although an orally administered vaccine may only need only be free of enteric pathogens).
- Vaccine manufacturers follow tightly regulated procedures (e.g. Good Manufacturing Practice; GMP) to ensure that vaccines are manufactured safely and consistently.
- Wherever possible they avoid the use of materials of human or animal origin.
- The acceptable safety of a vaccine may depend on the recipient. An adult choosing to receive a therapeutic anti-cancer vaccine may be prepared to accept a higher level of risk than a parent taking an infant for routine paediatric vaccination.



Phase 1 trials entail close clinical monitoring of vaccinees. Data collected might include information on the following: local and systemic reactions, fever, diarrhoea and vomitting and headache etc.

Phase 2 trials provide an opportunity to investigate the effect of different dose regimes and formulations, examine laboratory assays for correlates/surrogates of protection, and for regulatory laboratories to evaluate the prospective vaccine and validate QC tests. All groups that are likely to use the vaccine include both sexes, a range of ages and different ethnic groups.

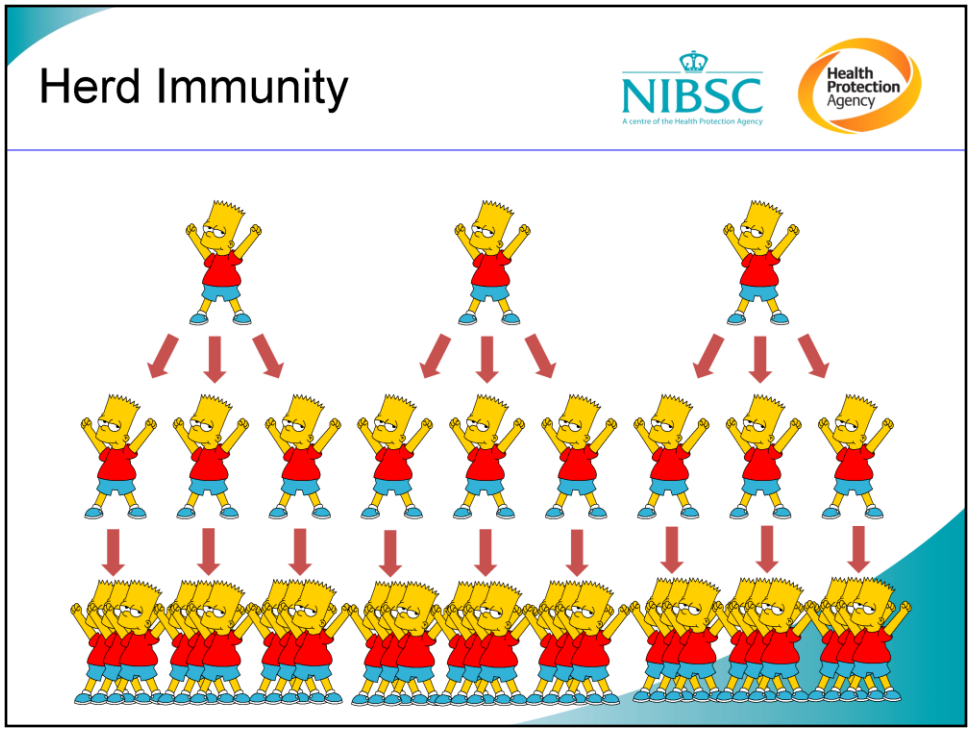
Phase 3 trials are designed to investigate directly the ability of a vaccine to offer protection against disease. This requires good disease surveillance. Efficacy is the measure of a vaccine to offer protection. Its assessment is scientifically rigorous (e.g. assessed by double blind, placebo controlled trial) and it is required for licensure. Effectiveness studies, sometimes termed phase 4 trials, measure the ability of the vaccine to achieve specific ends. They are scientifically less rigorous, study designs vary and they are not required for licensure. Effectiveness data can help to convince prospective users of the benefits of a vaccine.

Vaccine efficacy

- Vaccine efficacy is determined in Phase III trials
  -blinded, placebo controlled

$$\text{Vaccine efficacy} = 1 - \frac{\text{Attack rate in vaccinated group}}{\text{Attack rate in unvaccinated group}}$$

- Usually expressed as a percentage

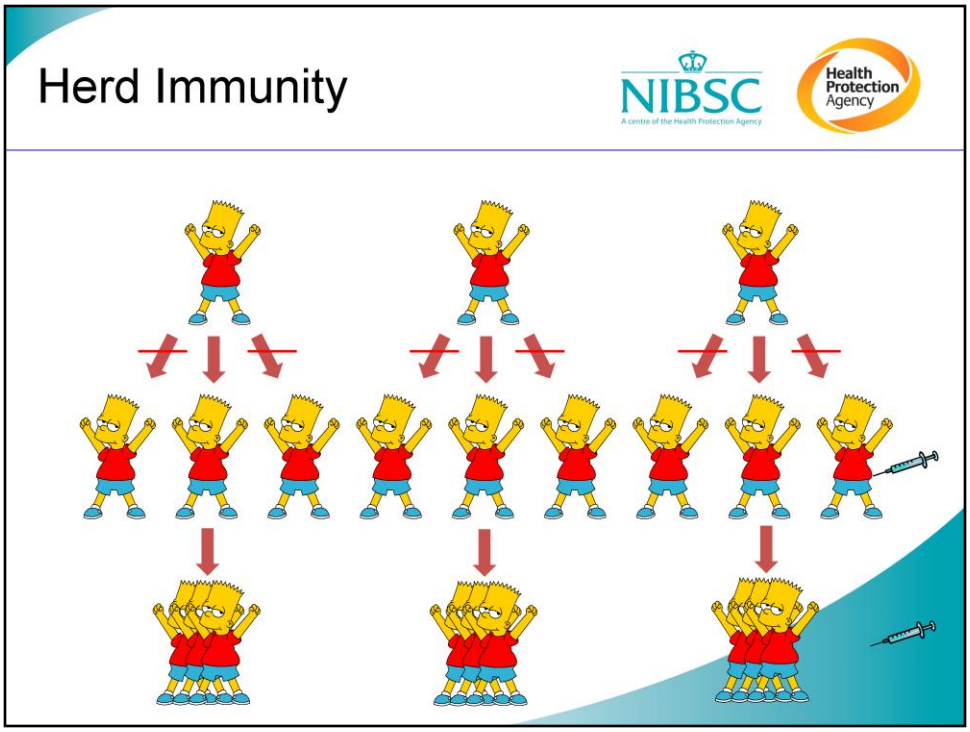


Herd immunity describes the situation when the vaccination of a portion of the population provides protection to unvaccinated individuals

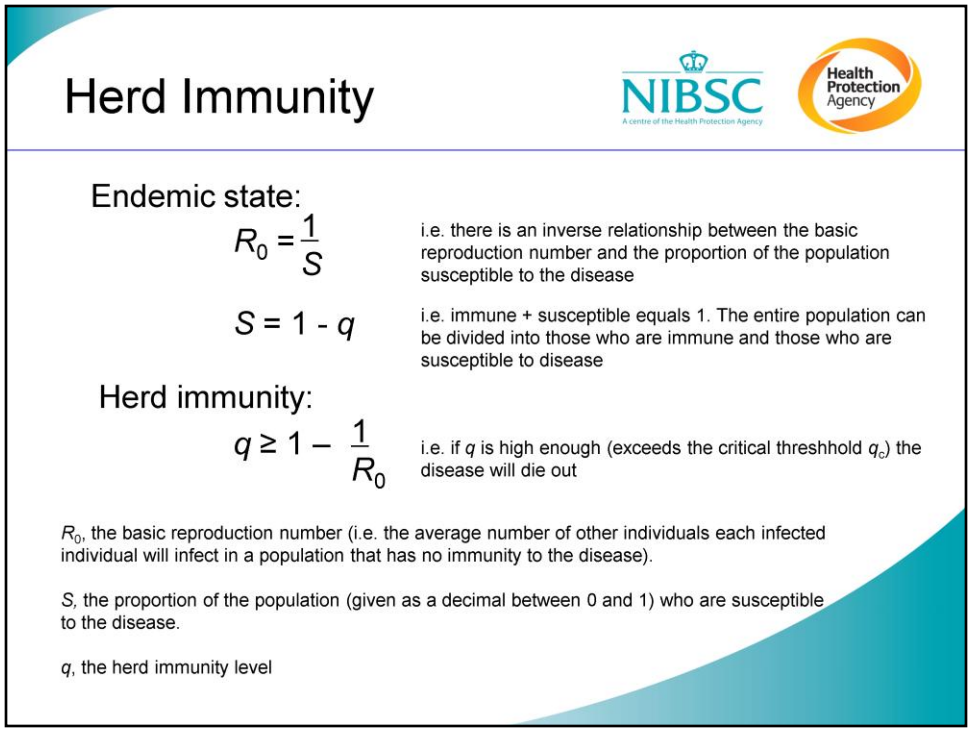
Herd immunity works by disrupting the transmission of a pathogen in the population and is not relevant when an individual needs protection independently of the population e.g. Travellers

The impact of herd immunity in vaccine programmes should not be underestimated. Herd immunity is particularly important for those who are unable to be vaccinated (e.g. the immunocompromised).

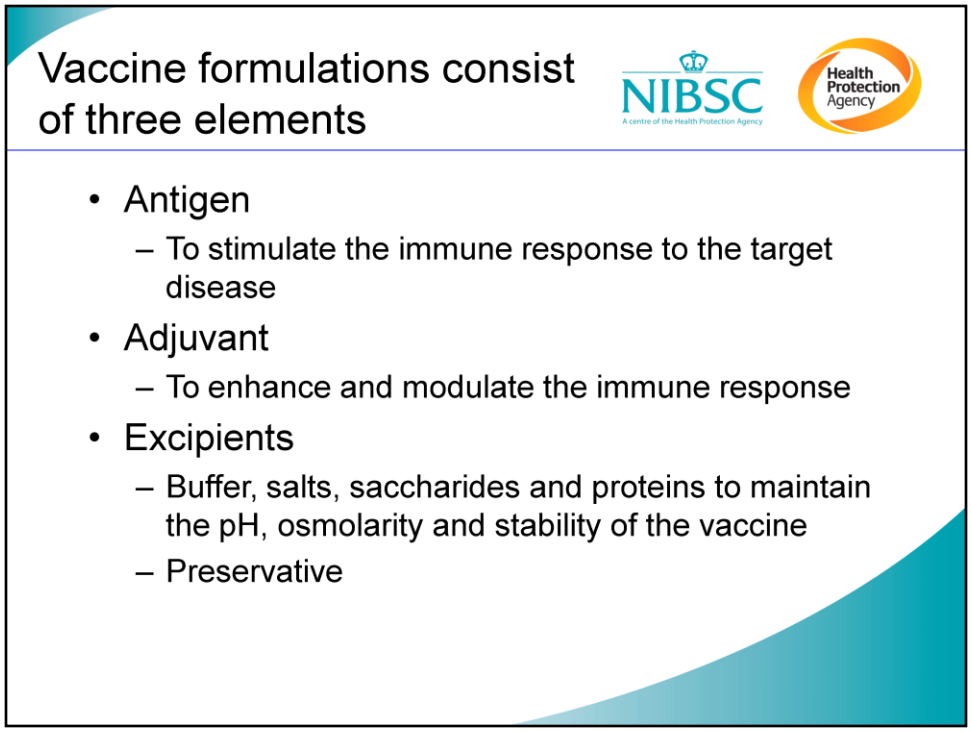
This is a model disease with a reproduction number of 3. The organism spreads rapidly through the population.



Here a vaccine has been introduced with an effectiveness of 67% against transmission. Even if the next level of infected individuals are unimmunised there is a marked reduction in the number of carriers. If this level is also immunised you will see a reduction in carriage and hence disease.

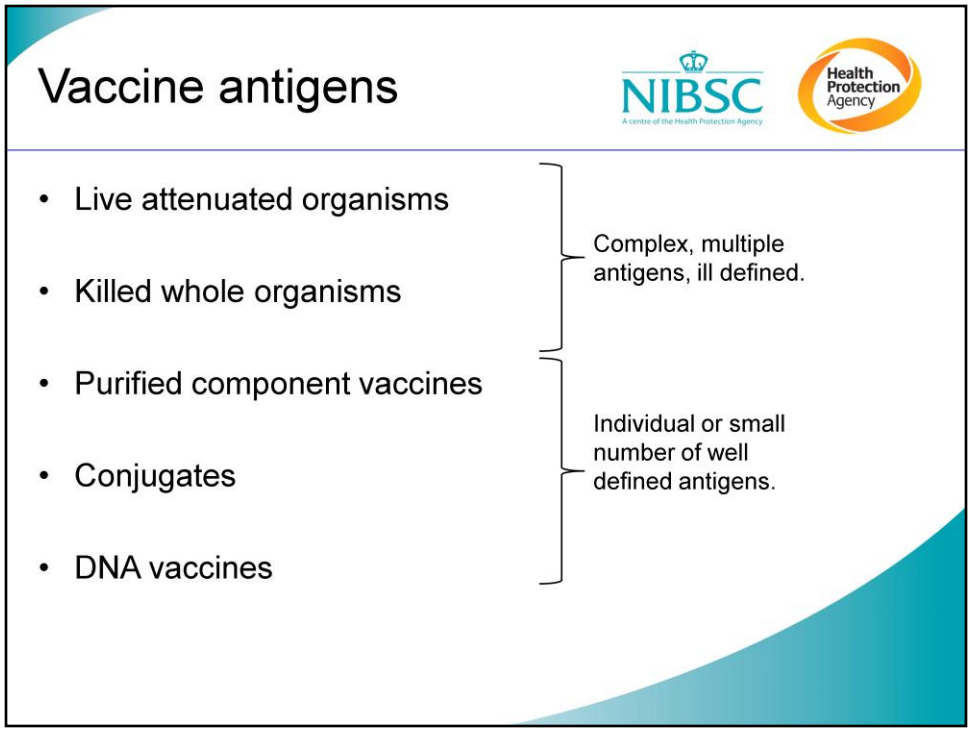


The term herd immunity refers to the effect of immunity within a population to reduce transmission of the infectious agent, thereby protecting those who are susceptible (i.e. not immune) to the disease. The endemic state reflects a balance between the transmissibility of the infectious agent and the level of immunity in the population. Herd immunity can be modelled mathematically and this slide shows this in a simplified form. q_c is the minimum proportion of the population that must be immunised at birth (or close to birth) in order for the infection to die out in the population.



The constituents of a vaccine formulation can be divided into three categories: the antigen(s), the adjuvant and other excipients.

Preservatives such as phenoxyethanol or the mercury containing Thiomersal may be added to multidose formulations to prevent growth of microbes once the vial has been punctured. Single dose vials or pre-filled syringes do not usually contain preservative.



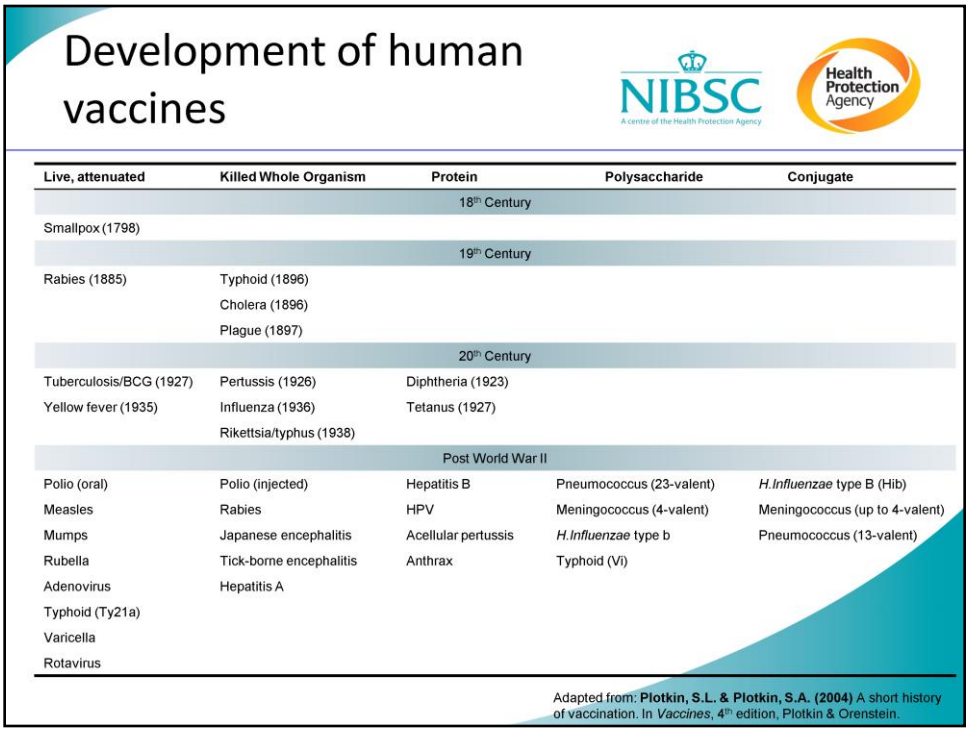
The antigenic components of vaccines can be divided into four categories.

Live attenuated organisms. These contain mutations that affect the ability of the organism to thrive and/or cause disease in the host. Historically, the mutants were isolated by chemical mutageneisis or multiple passaging of the organism; more recently attenuated isolates have been rationally mutated using targetted molecular methods.

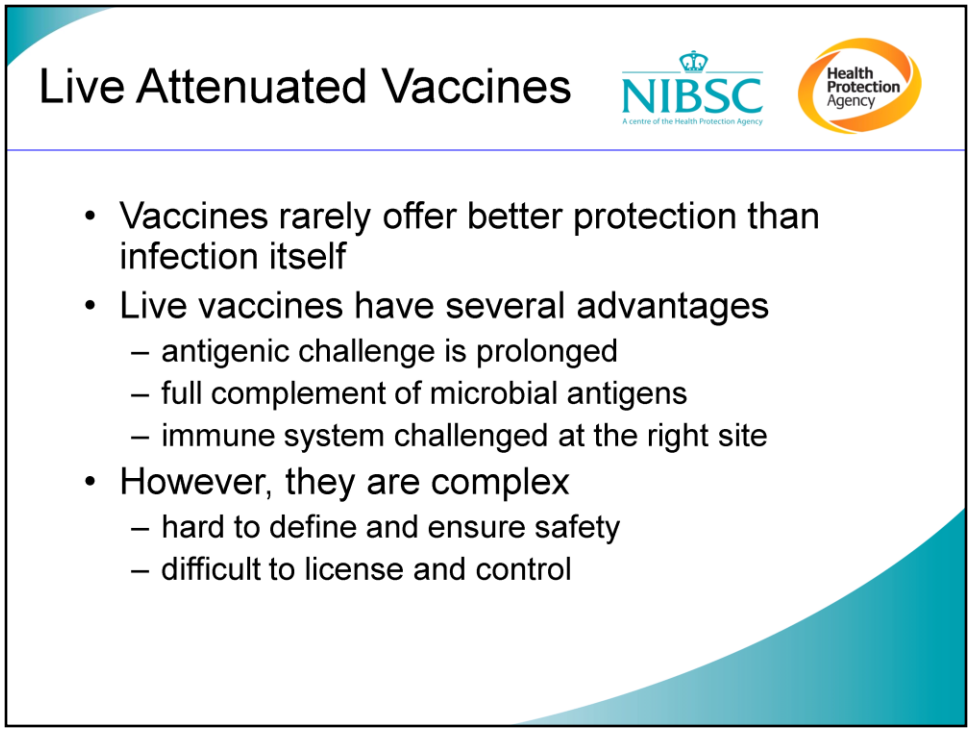
Killed organisms. Probably the simplest sort of vaccine to produce. The organism is grown and then killed either chemically (e.g. with phenol or formaldehyde) or by heating.

Component vaccines. The emergence of purified component vaccines has depended upon technological advances since the latter part of the 20th century. These have included advances in physical and chemical methods of separation as well as the development of recombinant DNA techniques, genome sequencing and bioinformatics (for the identification of prospective antigen genes in the genome).

DNA vaccines. The antigen gene is cloned in a vector so that it is expressed from a promoter sequence that is functional in the host. Once the DNA is injected, the host expresses the desired antigen and then mounts an immune response.



There has been a marked increase in the number of vaccines for the prevention of infectious diseases. This has been characterised by an increasing number of purified protein and saccharide component vaccines. Recently, there has been an increase in the number of licence submissions of vaccine candidates developed using molecular genetic methods, reflecting the technological changes that have taken place in microbiology during the last three decades.



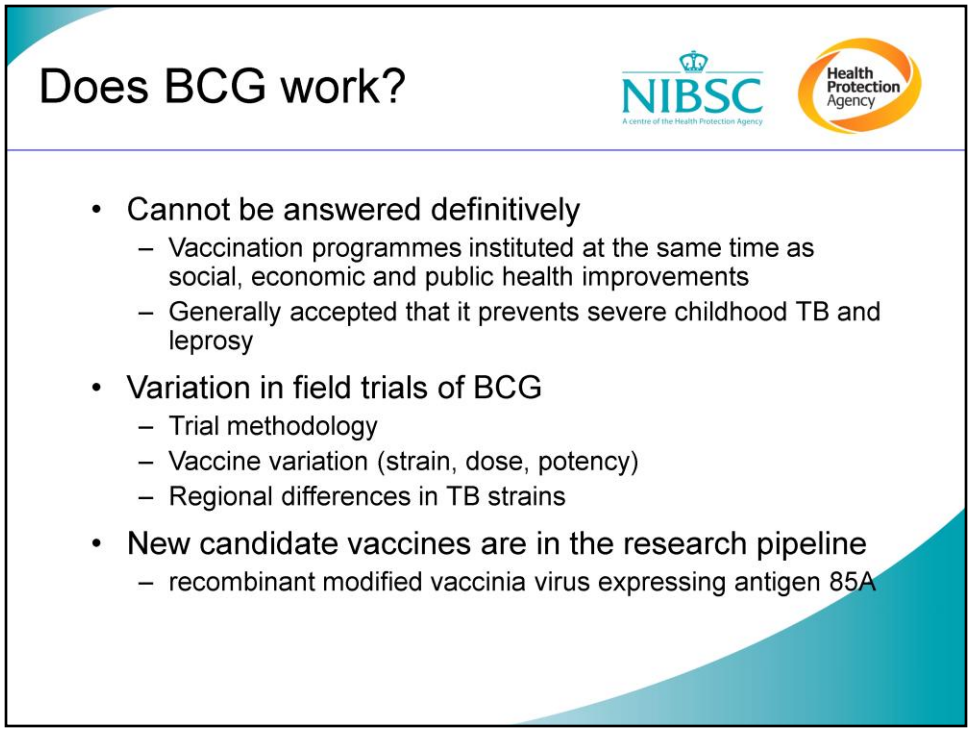
The pros and cons of attenuated vaccines.

Vaccination against tuberculosis (BCG)

- Attenuated strain of *Mycobacterium bovis*.
  - Serial passage, 231 times over 13 years (1908-1921)
  - Phenotypic changes: rough/dry to moist/smooth
  - Genotypic changes: loss of RD1 region encoding 9 proteins

Albert Calmette (1863-1933)

Camille Guérin (1872-1961)



Evidence for the effectiveness of BCG vaccine is equivocal. As a result, there are a number of candidate vaccines in the R&D pipeline. Arguably the most advanced of these, in terms of clinical development, is the recombinant modified vaccinia virus Ankara (smallpox vaccine) expressing a major secreted antigen from Mycobacterium tuberculosis, antigen 85A.

Typhoid Vaccine (Vivotif ®)

- Current typhoid vaccine based on strain Ty21a
  - *galE* mutant of *S. typhi* Ty2
  - isolated by NTG mutagenesis
  - formulated in enteric-coated capsules
  - safe
  - best efficacy data from school age children in Santiago, Chile shows three doses given on alternate days gives 67% (47-79%) efficacy.

Typhoid, or enteric fever, is caused by the bacterium *Salmonella enterica* servar typhi. It is transmitted by the ingestion of faecally contaminated food or water. The bacteria invade the intestinal wall and are taken up by macrophages. *S. typhi* is adapted to survive within the macrophage, which renders them resistant to damage by the immune response. In this form it is then spread throughout the body via the lymphatics. Typhoid fever is characterised by a sustained fever, profuse sweating, gastroenteritis, and non-bloody diarrhoea. It is a major global health problem causing an estimated 16-32 million cases annually and up to half a million deaths in endemic regions.

The live attenuated typhoid vaccine was developed using “old” technology, i.e. chemical mutagenesis. It is taken orally which ensures a good immune response in the gut. The organisms are lyophilised in enteric coated capsules allowing them to pass safely through the low pH environment in the stomach. The capsules are acid resistant but dissolve readily at neutral pH.

Killed Whole Cell Vaccines

- Simple to manufacture
  - harvest culture and kill (e.g. formaldehyde, heat etc)
  - large scale
- Examples
  - pertussis, cholera, plague
- Variable efficacy
- Complex and ill defined
  - often reactogenic because of high endotoxin content
- Contamination with culture constituents
- Immune response complicated to define
- Difficult to license and control

Killed Whole Cell Cholera Vaccine

- Killed whole cell parenteral vaccine
  - poor efficacy
  - short-lived protective response
  - strain specific
- Killed whole cell oral vaccine
  - good efficacy in cholera endemic area (Vietnam trials)
  - oral delivery
  - safe
  - >85% show >4-fold rise in vibriocidal antibodies (<3% in controls)
  - protective efficacy 66% after two doses
  - WHO mediated technology transfer
- Dukoral™
  - killed whole cell + CtxB oral vaccine
  - drink formulation (3 doses at weekly intervals, booster every 2 years)
  - may also protect against ETEC

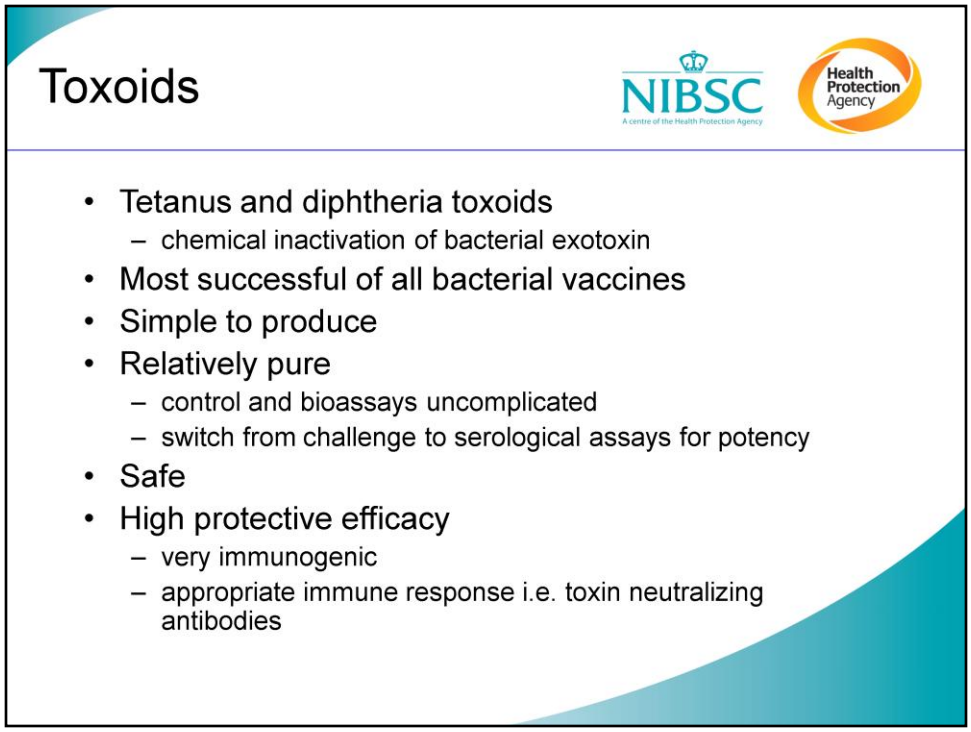
The first cholera vaccine, developed in the 1920s, was a killed organism preparation, which was highly reactogenic and had questionable efficacy. It was largely discredited in a number of reports during the latter part of the 20th century and the WHO recommendation for its use was withdrawn in the early 1990s.

Recently, an orally administered version of the vaccine has proved to be safe and more efficacious in studies in the Far East. This vaccine has been produced in Vietam following a technology transfer agreement with a Swedish company, SBL. The version of the vaccine produced in Sweden also contains recombinant B subunit of the enterotoxin. As this is immunologically cross-reactive with enterotoxin produced by some strains of *E. coli* (ETEC), there is some evidence to suggest that this vaccine also offers protection against diarrhoea cause by ETEC.

Subunit or Component Vaccines (1)

Painting by Sir Charles Bell

- Toxoids
  - the pathogenesis of some diseases depends largely or entirely on the action of bacterial exotoxins (tetanus, diphtheria, botulism)
  - toxin neutralizing antibodies can be sufficient for protection
  - vaccines consisting simply of detoxified toxins (toxoids) in this case are effective



Tetanus toxin is a neurotoxin causing muscle spasm. It consists of light and heavy chains (MW 10k and 50k respectively). The light chain is an endopeptidase that cleaves a membrane protein of synaptic vesicles. The toxoid used in vaccines is produced from filtered culture supernatant, which is treated with 40% formaldehyde at 37^{o}C.

Diphtheria toxin like tetanus toxin is an exotoxin. It is a polypaptide with a MW of 58k and is secreted as a proenzyme which is cleaved into two fragments. Fragment B is responsible for attachment to and penetration of the host cell; Fragment A is the toxic moiety inhibiting protein synthesis and hence causing cell death. Like tetanus toxoid, diphtheria toxoid is produced by formaldehyde treatment of culture supernatant.

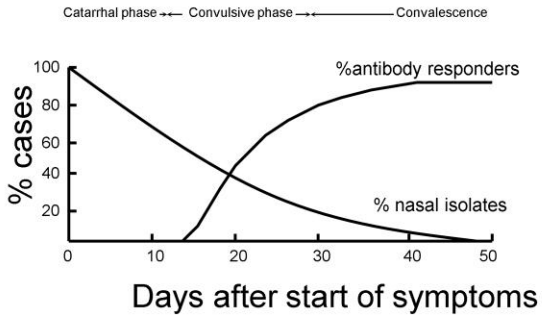
More recently toxoids have been developed for the exotoxins including those produced by *Clostridium difficile* and *Pseudomonas aeruginosa* though these have yet to be licensed by the regulatory authorities.

Subunit Vaccines (2) - cell surface components

Immune system first mounts antibody response to the surface antigens of the organism

- Proteins
  - diverse (porins, pili, flagella etc)
  - good immunogens
  - usually safe
  - may be antigenically variable
  - may be phase variable
- Carbohydrates
  - capsules and endotoxin
  - T cell independent immune response

Whooping Cough



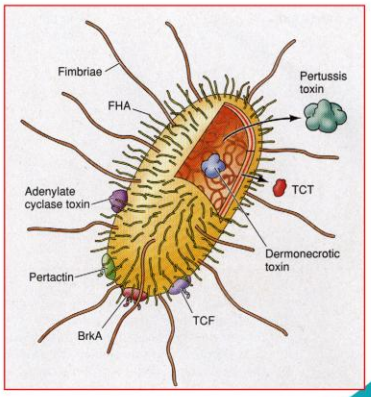
- Caused by *Bordetella pertussis* infecting ciliated epithelium and releasing toxin
- Condition identified by characteristic convulsive cough
- Recovery coincides with antibody production

Acceptibility of whole cell pertussis vaccine

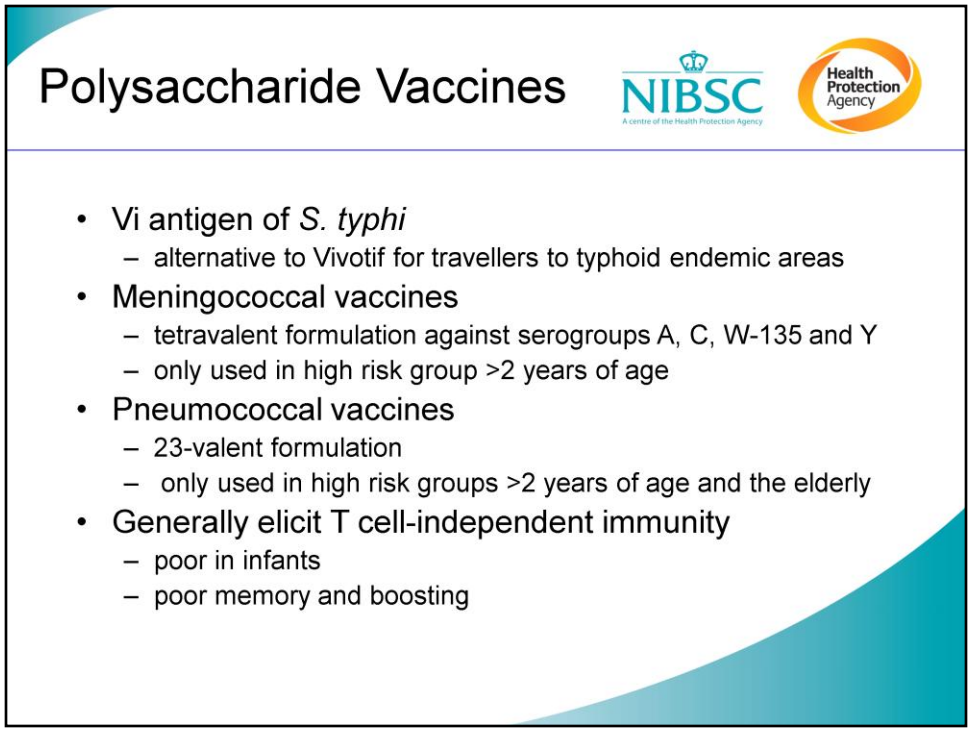
- Whole cell pertussis vaccine is effective
  - efficacy rates typically >90%
- It has been associated with a number of adverse reactions
  - anaphylaxis
  - prolonged crying
  - febrile siezures
  - acute encephalopathy (now known to be due to mutations within a sodium channel gene)
- Development of acellular (component) vaccine driven by poor acceptance of the whole cell vaccine

B. pertussis components associated with virulence

- Attachment to ciliated epithelium
  - filamentous haemagglutinin (FHA)
  - fimbriae
  - pertactin
- Adherence and complement resistance
  - BrkA
- Toxins
  - pertussis toxin (PT)
  - adenylate cyclase
  - tracheal cytotoxin
  - heat labile toxin
  - endotoxin



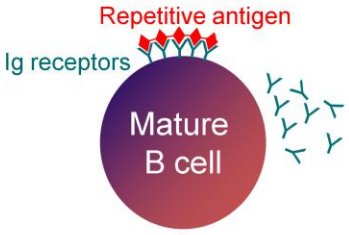
- Acellular vaccines
  - multicomponent (bi-,tri- and pentavalent fromulations)
  - pertussis toxin, FHA, pertactin, fimbrial antigens 2 and 3
  - safe and efficacious (75-90%)



Some bacteria produce polysaccharide capsules. These bacteria include organisms that colonise the nasopharynx, such as *Streptococcus pneumoniae, Haemophilus influenzae* and *Neisseria meningitidis*, which occasionally cause invasive infections like septicaemia and meningitis. Although these bacterial species produce a wide range of immunochemically distinct capsules, only certain capsular types are associated with disease indicating that the capsule plays a role in the virulence of these bacteria.

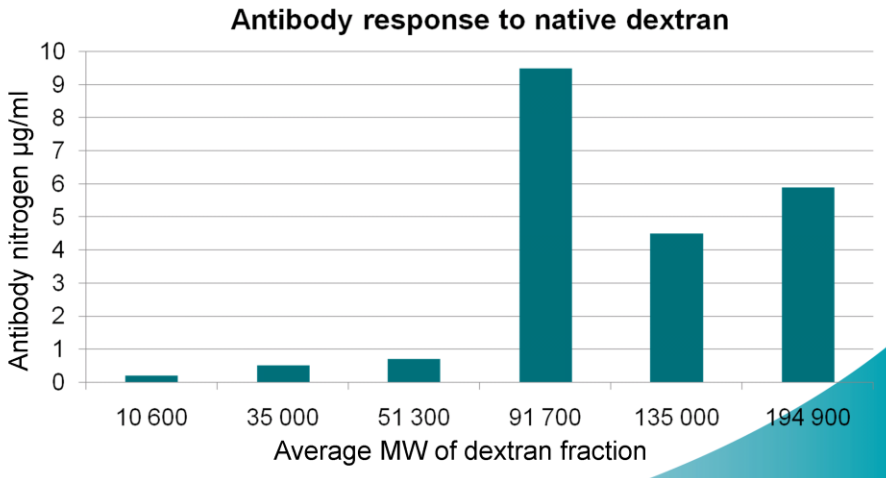
T cell independent antigens

- The Antigen
  - Usually large and linear
  - Not readily degraded
  - Highly repetitive determinant
- The Immune Response
  - Predominantly IgM
  - Poor memory effect
  - Low avidity antibody

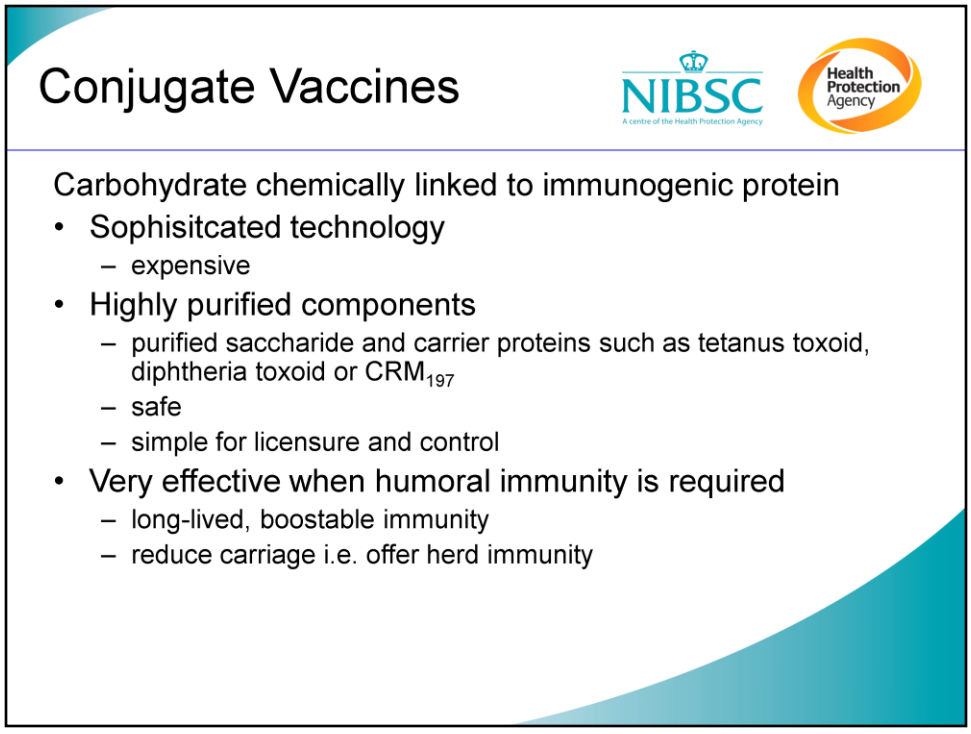


T cell independent antigens like polysaccharides activate mature B cells, in the absence of antigen presentation, by cross-linking immunoglobulin receptors on the cell surface. The resulting immune response is not ideal for vaccination. It is typically characterised by poor immunological memory, low avidity antibodies (no affinity maturation) that are less likely to offer functional protection against disease, and in many cases repeated doses rather than boosting can lead to immunological hyporesponsiveness.

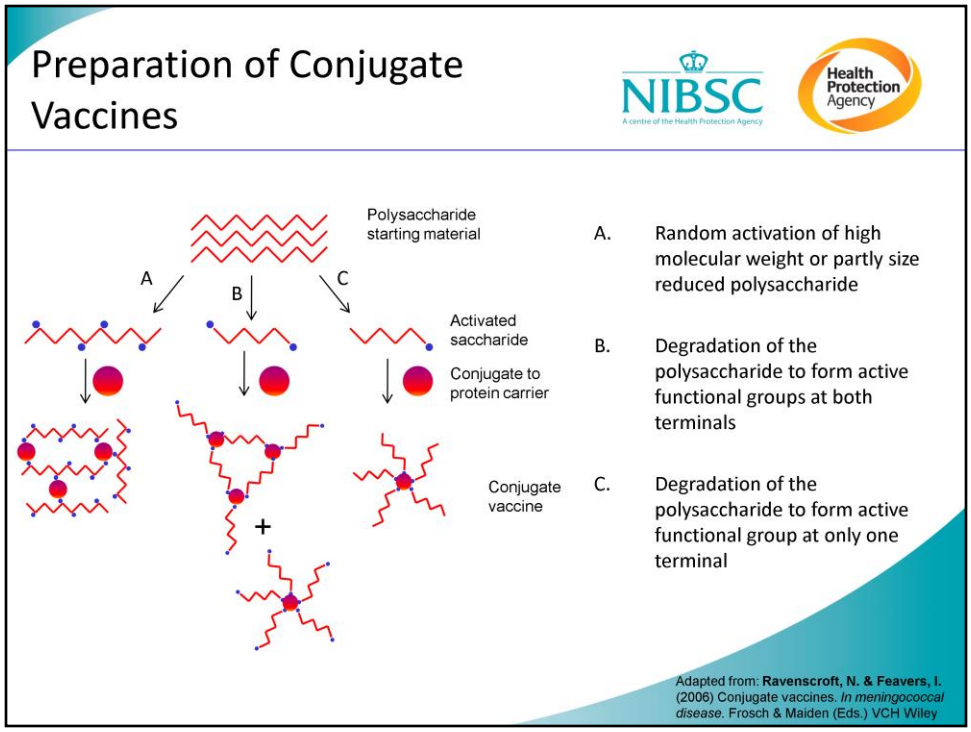
Size matters for T cell independent polysaccharides



Kabat and Bezer (1958) The effect of variation on molecular weight on the antigenicity of dextran in man. Arch. Biochem. 78: 306-313



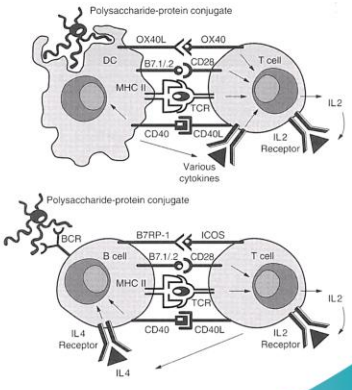
Conjugate vaccines, in which the saccharide moiety is chemically linked to a protein carrier, overcome the drawbacks of plain polysaccharide vaccines by making them T cell dependent.



Various approaches have been taken to the preparation of conjugate vaccines. The molecular structure of the vaccine depends on the chemistry used for conjugation and the ratio of saccharide to conjugate.

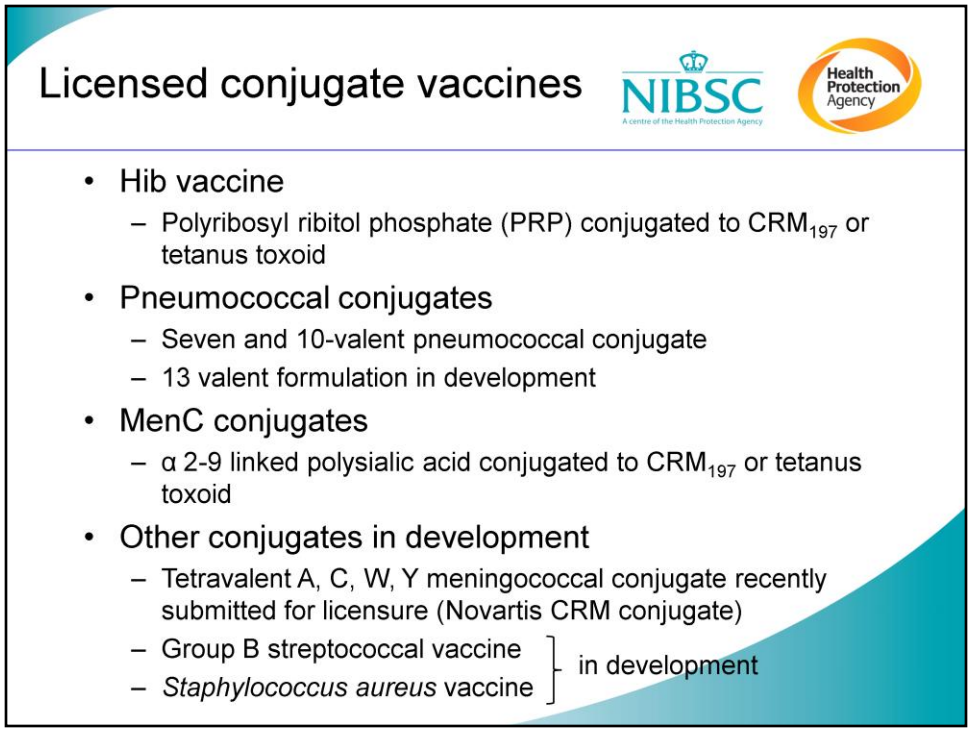
T cell dependent antigens

- Naive T cells primed by interaction with antigen presenting cells
  - Requires antigenic stimulation via MHCII-TCR pathway
- After priming T cells interact with B cells
  - Saccharide specific B cells take up conjugate and present the carrier peptides to T cells via MHCII
- Activated B cells differentiate into antibody secreting plasma cells and memory B cells



Adapted from Guttormsen *et al.* (1999) Infect. Immun. 67: 6375-6384

Conjugate vaccines interact with the cells of the immune system, more like proteins than carbohydrates, to elicit T-cell dependent immunity.



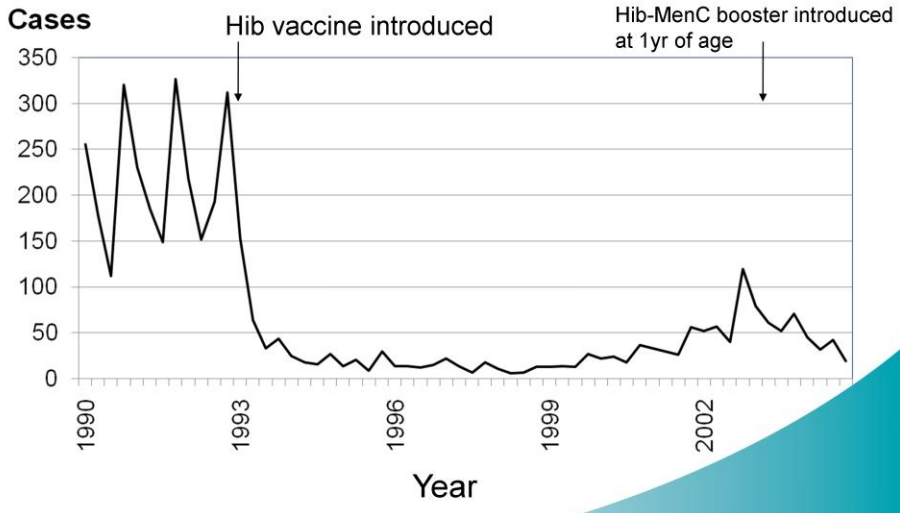
Hib vaccines are usually offered in combination with other paediatric components – DTaP and polio.

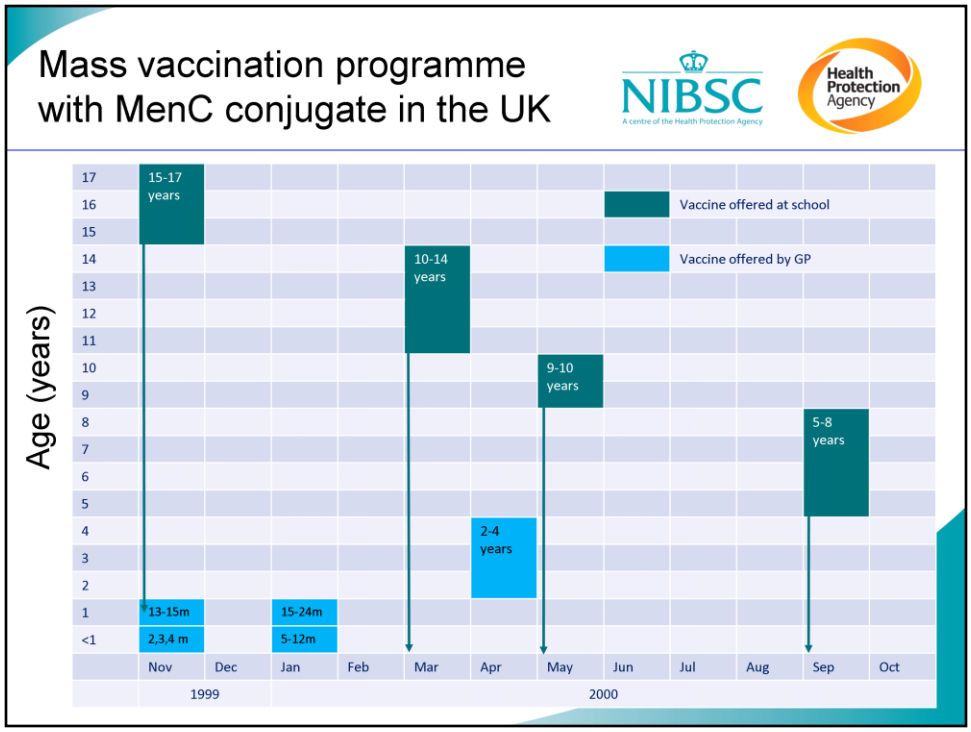
There are over 90 serotypes of peumococci, many of which cause disease. The first conjugateto be licensed by Wyeth Vaccines (now part of Pfizer) offered protection against 7 serotypes (4, 6B, 9V, 14, 18C, 19F, 23F). This vaccine is known as Prevenar. This year GSK has licensed a 10-valent pneumococcal conjugate (serotypes 4, 6B, 9V, 14, 18C, 19F, 23F plus 1, 5, 7F) in which the carrier protein is an antigen, known as protein D, from the surface of non-typeable *Haemophilus influenzae*. This vaccine is known as Synflorix. Wyeth currently has a licence application under consideration at the FDA and the EMEA for a 13-valent version of Prevenar (4, 6B, 9V, 14, 18C, 19F, 23F plus 1, 3, 5, 6A, 7F, 19A).

There are four versions of MenC conjugates licensed in Europe: Meningitec (Pfizer), Menjugate (Novartis), NeisVac (Baxter) and Menitorix (bivalent Hib/MenC booster, GSK).

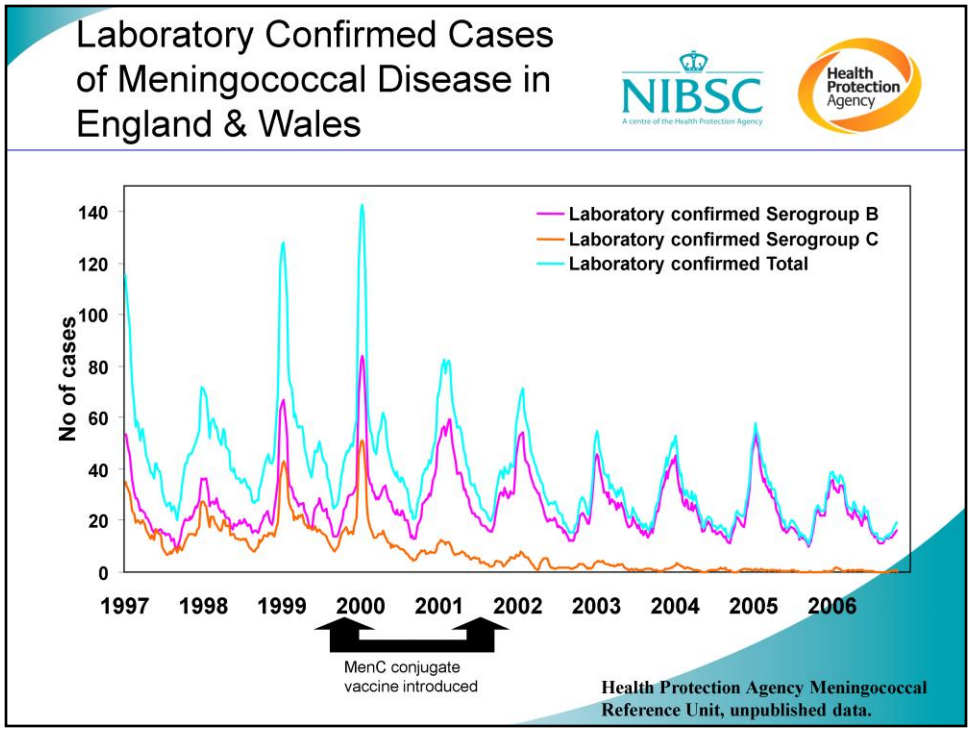
The conjugate vaccine approach has arguably been one of the most succssful developments of the late 20th century and many other conjugate vaccines are under development.

Immunity to *Hi*b

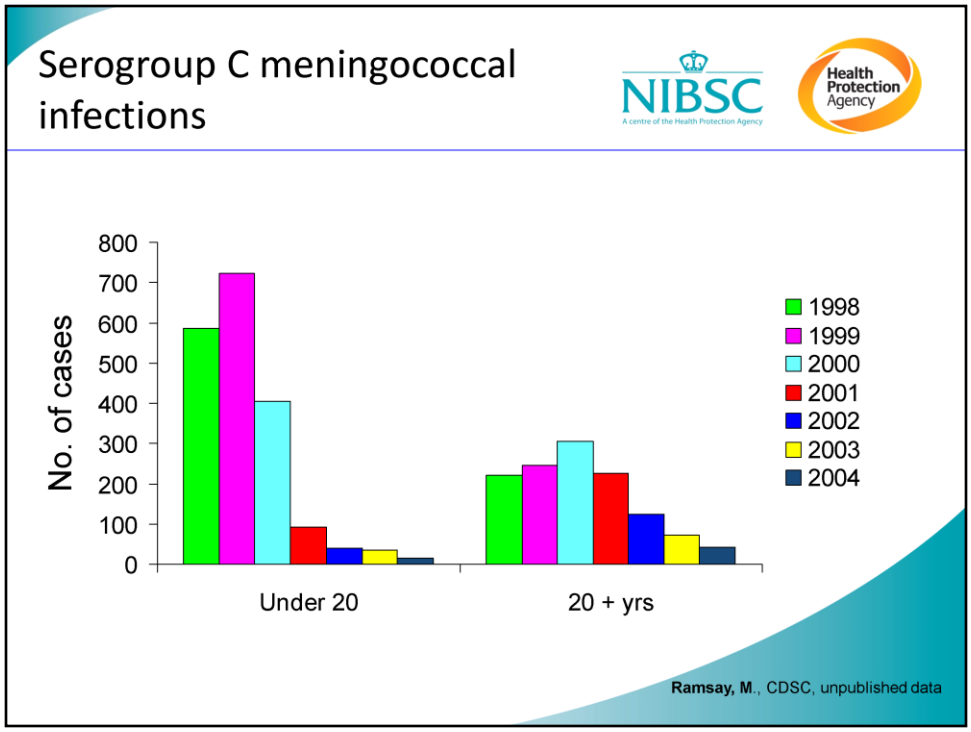




This shows how the vaccine was rolled out in the UK as increasing numbers of doses became available. The programme started with the groups most at risk of infection and ended with those least at risk. For those between the ages of 5 and 18 the vaccine was administered at school or college. The preschool children were vaccinated at the local surgery.



This figure shows the 5 week moving average of the number of cases of meningococcal disease occuring in England and Wales between 1997 and 2007. Almost immediately after the roll-out of the MenC conjugate vaccine the number of cases of MenC disease, shown by the red line, started to decline. Since 2005 there has been almost no disease in the UK. Since the introduction of the vaccine almost all disease in the UK has been caused by serogroup B meningococci for which there is currently no vaccine.



The number of cases of serogroup C meningococcal disease was reduced dramatically in both the vaccinated and unvaccinated age groups. This suggested that the vaccine offered herd immunity.

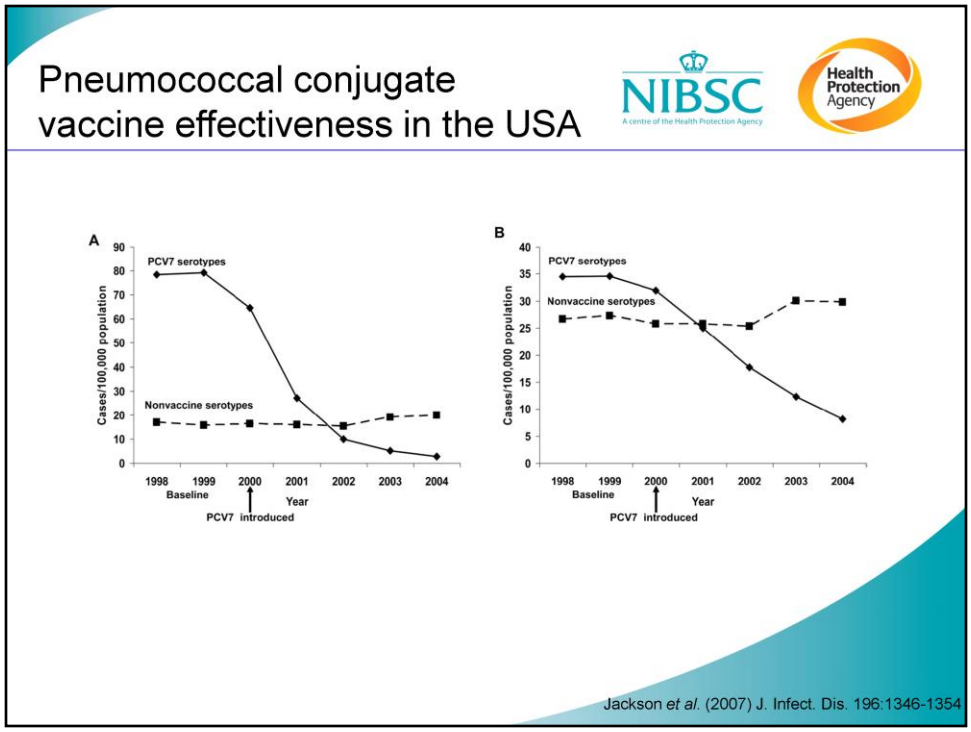
Pneumococcal conjugate vaccine efficacy: invasive disease and pneumonia

Double-blind study at Northern California Kaiser Permanente medical centres

Analysis for serotypes in vaccine	Cases Control:Vaccine	Efficacy (95% CI)	P
Fully vaccinated per protocol	39:1	97.4 (82.7-99.9)	<0.001
Intent to treat	49:3	93.9 79.6-98.5)	<0.001
Partially vaccinated	7:1	85.7 (0-100)	0.05
All cases regardless of serotype	55:6	89.1 (73.7-95.8)	<0.001

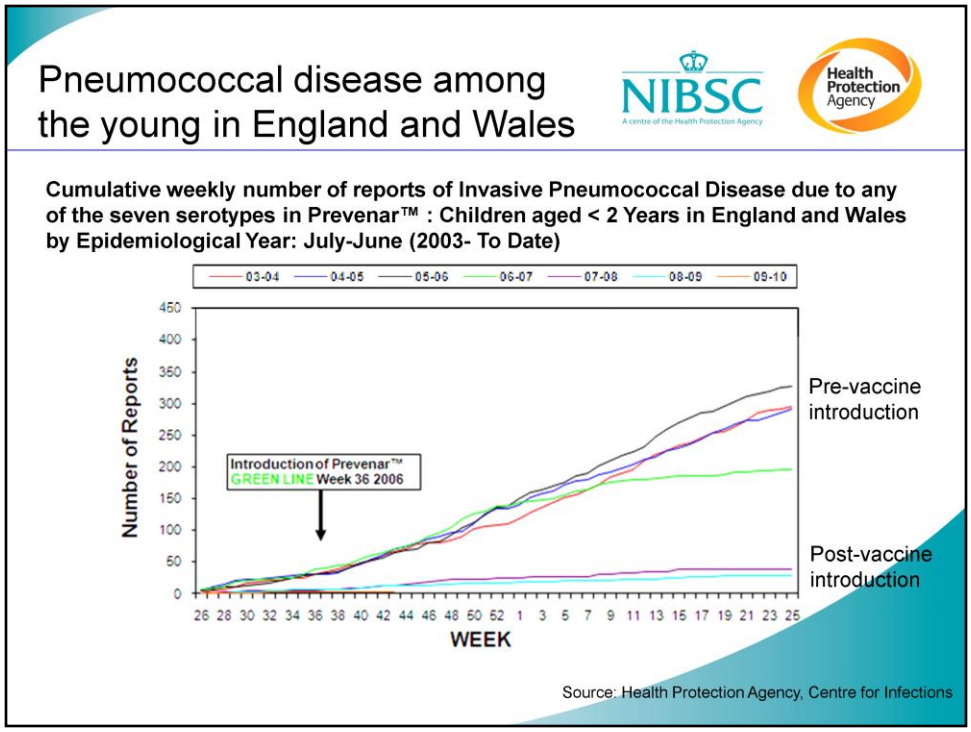
- Cases of pneumonia significantly reduced where there was a positive radiograph from 10.1 to 8.3 per 1000 person-years
- Estimated efficacy 17.7% (95% CI: 4.8 to 28.9) against pneumonia
- Reduction in cases of otitis media by 7-9%

Data from **Black *et al.*** (2000) Pediatr. Infect. Dis. J. 19: 187-195

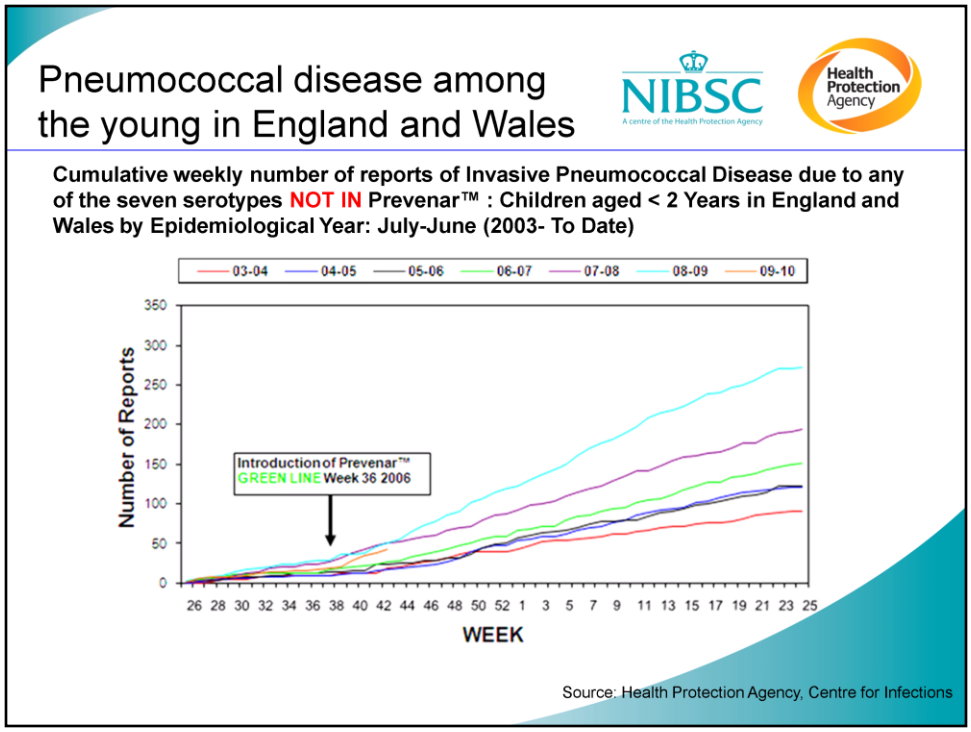


There have been numerous studies that reaffirm the effectiveness of the 7-valent pneumococcal conjugate vaccine since it was licensed in 2000. Rates of invasive pneumococcal disease among children

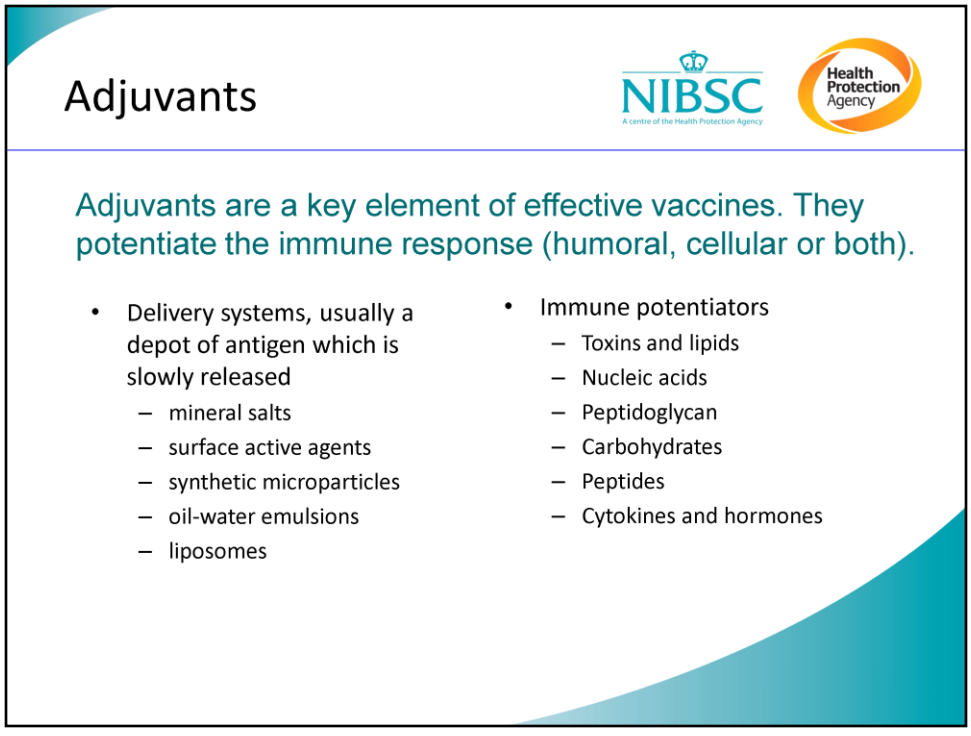
aged <5 years (A) and adults aged 65 years (B), by serotype and year. The 7-valent pneumococcal conjugate vaccine (PCV7) includes serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F. The annual incidence of disease due to nonvaccine serotypes increased from an average of 16.3 cases/100,000 population during prevaccine years (1998–1999) to 19.9 cases/100,000 population in 2004 for children aged <5 years and from 27.0 cases/100,000 population during prevaccine years to 29.8 cases/100,000 population in 2004 for adults aged 65 years. Significant increases in the incidences of disease due to serotypes 3, 15, 19A, 22F, and 33F were observed among children during this period; serotype 19A has become the predominant cause of invasive disease in children. In short, the incidence of pneumococcal disease caused by non-vaccine serotypes is increasing.



Cumulative curves of the cases of disease occurring across the year provide a convenient way of looking at the impact of a vaccination programme. The introduction of the 7-valent conjugate in the UK caused a dramatic reduction in invasive pneumococcal disease caused by the serotypes included in the vaccine. The green line represents the year in which the vaccine was introduced, the blue and purple lines the subsequent two years.

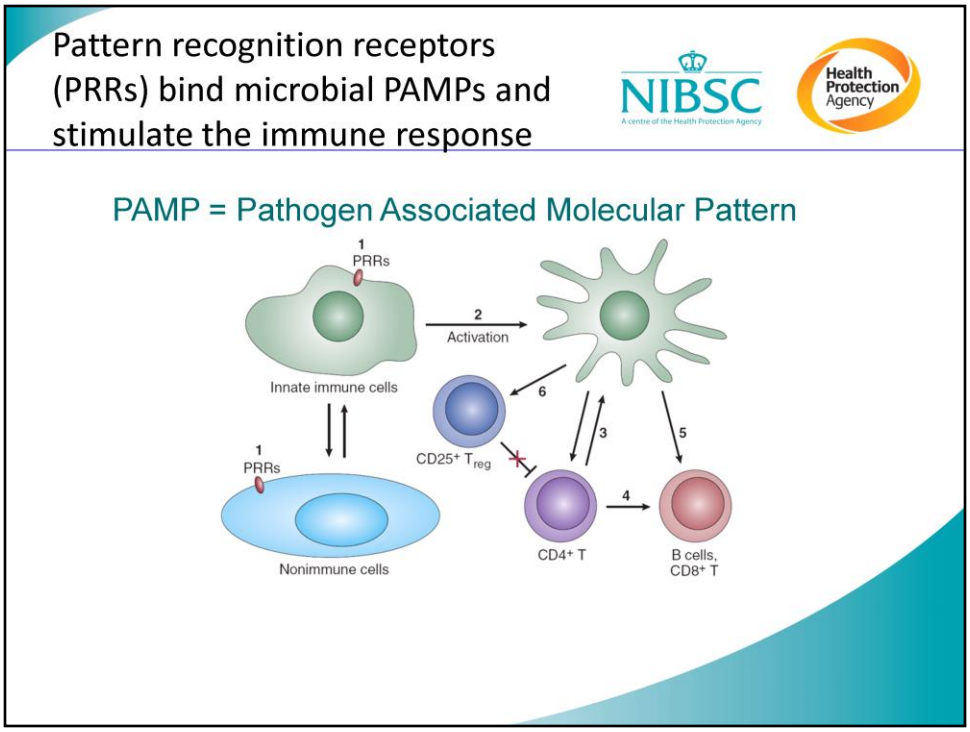


As expected the incidence of non-vaccine serotypes did not change immediately following the introduction of the 7-valent vaccine (green line). However, there has subsequently been a notable increase in invasive pneumococcal disease caused by the non-vaccine serotypes. The introduction of 10- and 13-valent formulations may address this problem.

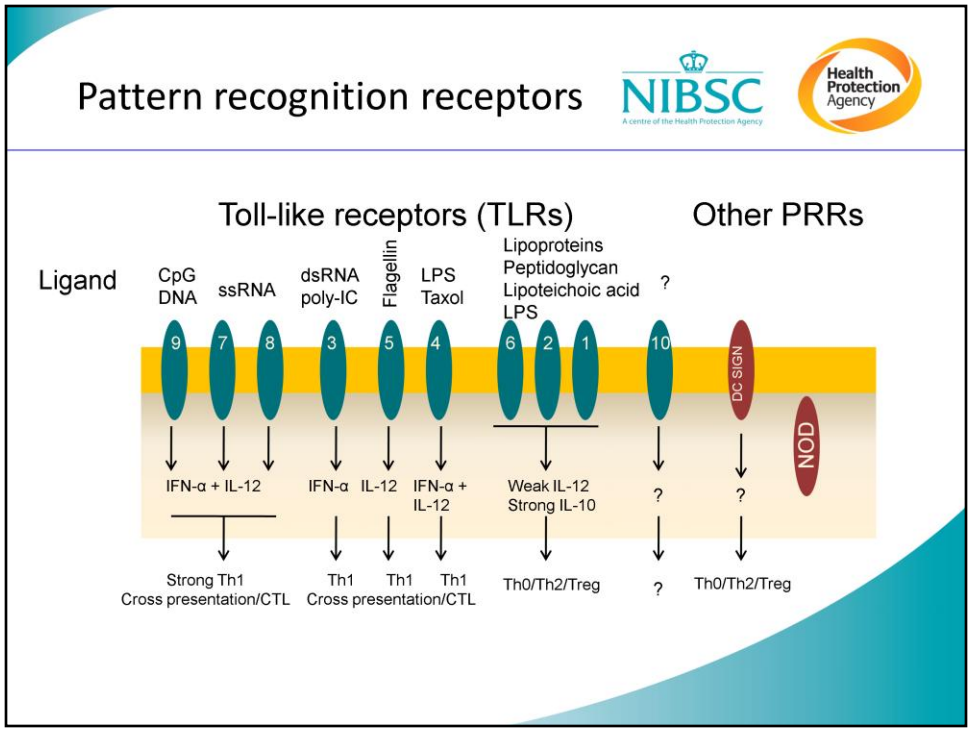


Adjuvants are not themselves the functional component of vaccines in the sense that the antigens are but they serve to potentiate and direct the immune response. They can serve one or a combination of roles. They might be used simply to enhance/strengthen the immune response, to determine whether the humoral or CMI arms of the immune system are stimulated, to direct the Th cell response, or to favour antibody subclasses.

Adjuvants broadly fall into two groups: delivery systems and immune potentiators. The former usually create a depot of antigen that can be released over a period to maximise the immune response. The latter specifically stimulate the immune system to obtain the desired response.



The immunostimulatory adjuvant components often contain PAMPs. These are recognised by receptors on cells of the innate immune system ultimately causing them to produce cytokines that influence the behaviour of other cells in the immune system. Adjuvants function primarily before an acquired immune response has been induced to influence the nature and potency of that response.

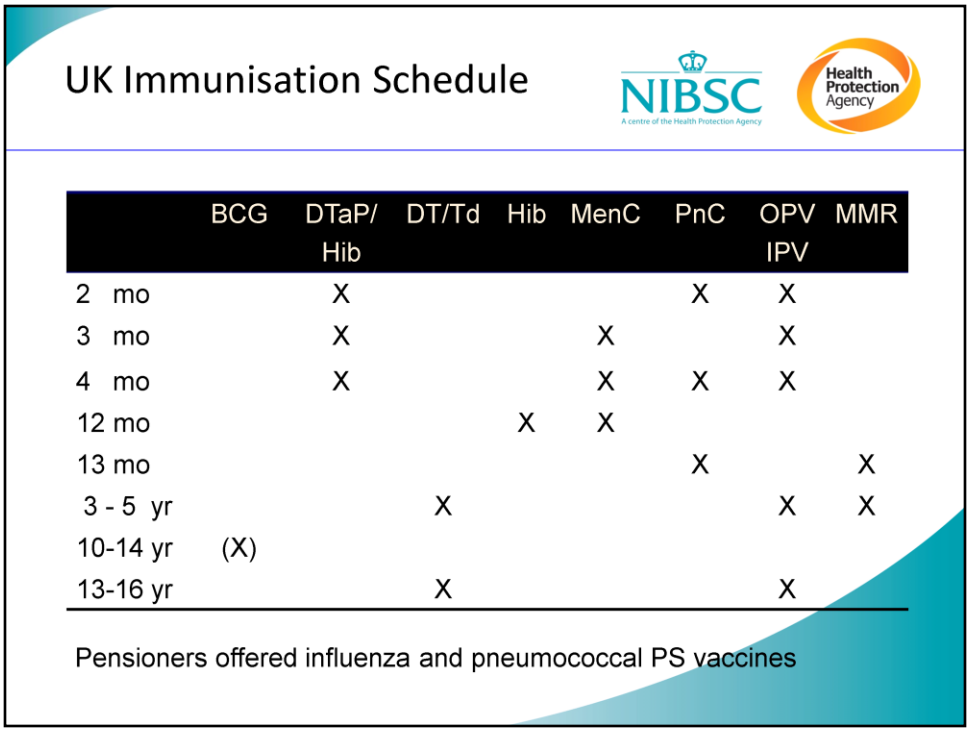


This slides provides some examples of PRRs on innate immune cells. Ligands that might typically associated with viruses (e.g.nucleic acids) stimulate interferon and IL-12 production which in turn drive a Th1/cytotoxic lymphocyte response. This would be appropriate for the elimination of a viral infection. Conversely, bacterial PAMPs such as LPS, lipoproteins and peptidoglycan stimulate IL-10 which drives a Th2/humoral type response, which is more appropriate for the elimination of bacterial infections.

Examples of adjuvants licensed for use in humans

Adjuvant type	Licensed Examples	Description	Vaccines
Mineral salts	Aluminium and Calcium salts	Long established adjuvant widely used in current vaccines	DTP, conjugates etc.
Emulsions and surfactant-based formulations	MF59	Microfluidized detergent-stabilized oil-in-water emulsion	Influenza vaccine
Particulate delivery systems	Virosomes	Unilamellar liposomal vehicles	Flu and HepA
	VLPs	Virus subunits	HPV vaccine
Microbial derivatives	MPL	Monophosphoryl lipid A	HPV vaccine

UK Immunisation Schedule



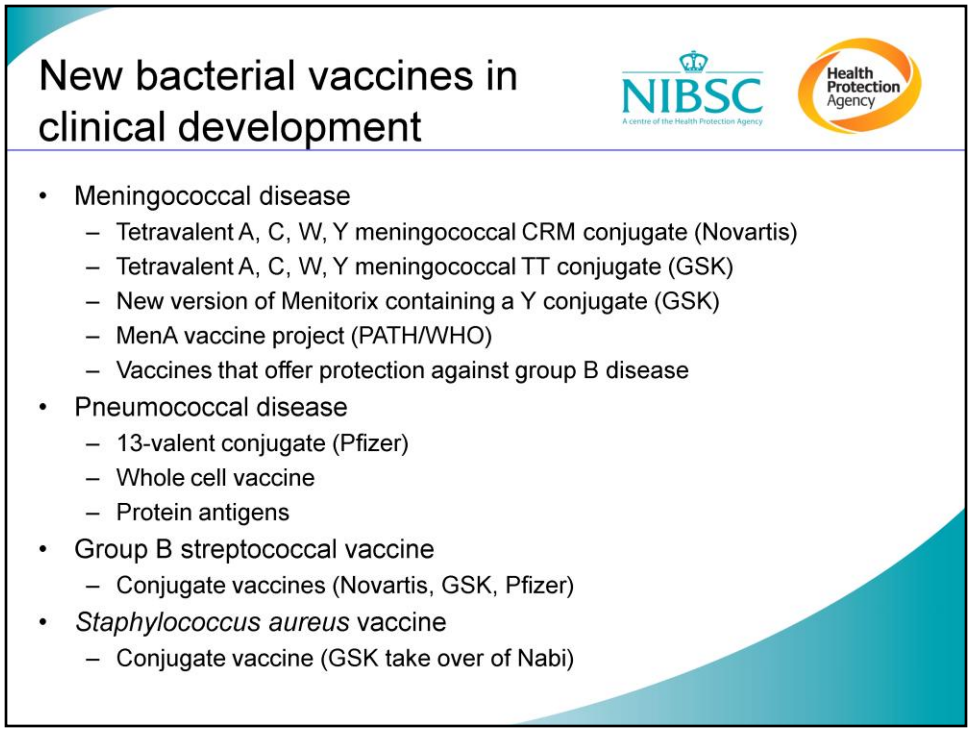
	BCG	DTaP/ Hib	DT/Td	Hib	MenC	PnC	OPV IPV	MMR
2 mo		X				X	X	
3 mo		X			X		X	
4 mo		X			X	X	X	
12 mo				X	X			
13 mo						X		X
3 - 5 yr			X				X	X
10-14 yr	(X)							
13-16 yr			X				X	

Pensioners offered influenza and pneumococcal PS vaccines

The routine immunisation programme is very crowded so that finding space for new vaccines is a challenge. Vaccine manufacturers are constantly looking for ways to combine components and thereby reduce the number of injections infants have to receive during the first few months of life. Currently in the UK the tetanus, diphtheria, inactivated polio and Hib components are given as single vaccine at 2, 3 and 4 months in a vaccine called Pediacel™ (Sanofi). Clinical trials have shown that the pneumococcal and meningococcal conjugates can be given effectively in a 2+1 strategy, i.e. 2 primary doses and a booster at about 12 months. BCG used to be offered to children at secondary school but today it is only offered to those seen to be at high risk.

Vaccinating the immunocompromised: a risk benefit analysis

- Need to protect the vulnerable patient
- Public health perspective (herd immunity)
- Protect the patient by immunising close contacts
- Live vaccines need particular consideration
- Decision on whether to vaccinate depends on the nature of the immunodeficiency and the vaccine



•Tetravalent meningococcal conjugates are likely to be used primarily for travellers to areas at high risk of MenA disease (sub-Saharan Africa, the Middle East and China).

•A version of Menitorix containing a Y conjugate (i.e. Hib-MenC-MenY) is aimed at the US market where there is more Y disease than in the UK. A recent carriage study among Nottingham University students has shown an increased level of serogroup Y carriage in the UK. If Y disease increases in the UK it may also be useful here.

•Group A meningococci cause large epidemics of disease in sub-Saharan Africa, among some of the poorest countries in the world. The MenA project, driven by PATH and the WHO, is to produce a vaccine that is affordable for this region. It relies on technology transfer to the Serum Institute of India where the vaccine will be produced at less than 50 cents a dose. Licensure of this vaccine is anticipated next year.

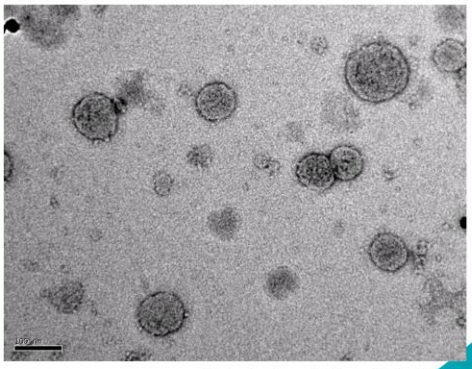
•Group B meningococcal disease remains a problem for vaccine developers. The group B polysaccharide, an α 2-8 linked polysialic acid, is similar to the glycosylation of some host cell-surface structures. It has proved poorly immunogenic, even when conjugated and has raised concerns that such a vaccine might elicit an anti-self response. Vaccine candidates based on protein antigens are well advanced in clinical trials.

•The serotype replacement problem associated with pneumococcal conjugate vaccines, together with a desire to find cheaper alternatives for developing countries, has lead various groups and companies to look at whole cell and protein antigen alternatives for the prevention of pneumococccal disease.

•Group B streptococci are responsible for a significant proportion of neonatal deaths. Several companies have conjugate vaccines at different stages of clinical development. As the infant acquires the organism from the mother at birth, these vaccines are likely to target females before or during pregnancy.

Meningococcal Outer Membrane Vesicle Vaccines

- OMVs are released spontaneously by meningococci *in vivo* and in culture

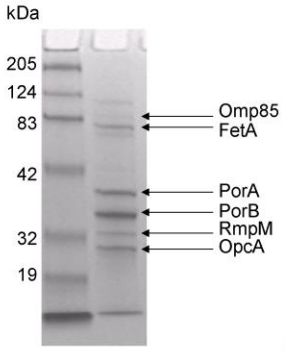


Picture by Anwen Bullen and Roland Fleck

The meningococcus is one of a small number of Gram –ve species that naturally bleb off outer vesicles of outer membrane. Just like the outer membrane they consist of lipopolysaccharide (LPS) and proteins. Vaccine is made by extracting the OMVs with detergent to reduce the LPS content and thereby make them less reactogenic. These vaccines are more complex than other purified component vaccines. Even with very careful manufacture the antigen profile and the LPS content can vary from batch to batch.

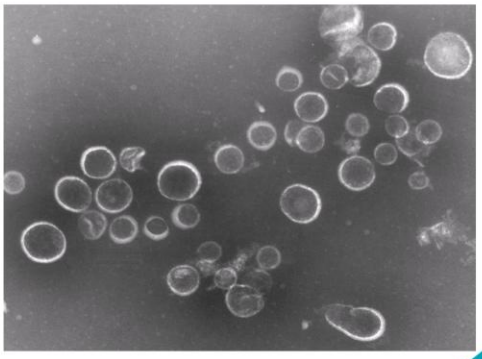
Meningococcal Outer Membrane Vesicle Vaccines

- OMVs are released spontaneously by meningococci *in vivo* and in culture
- They contain all the protein antigens usually associated with the OM

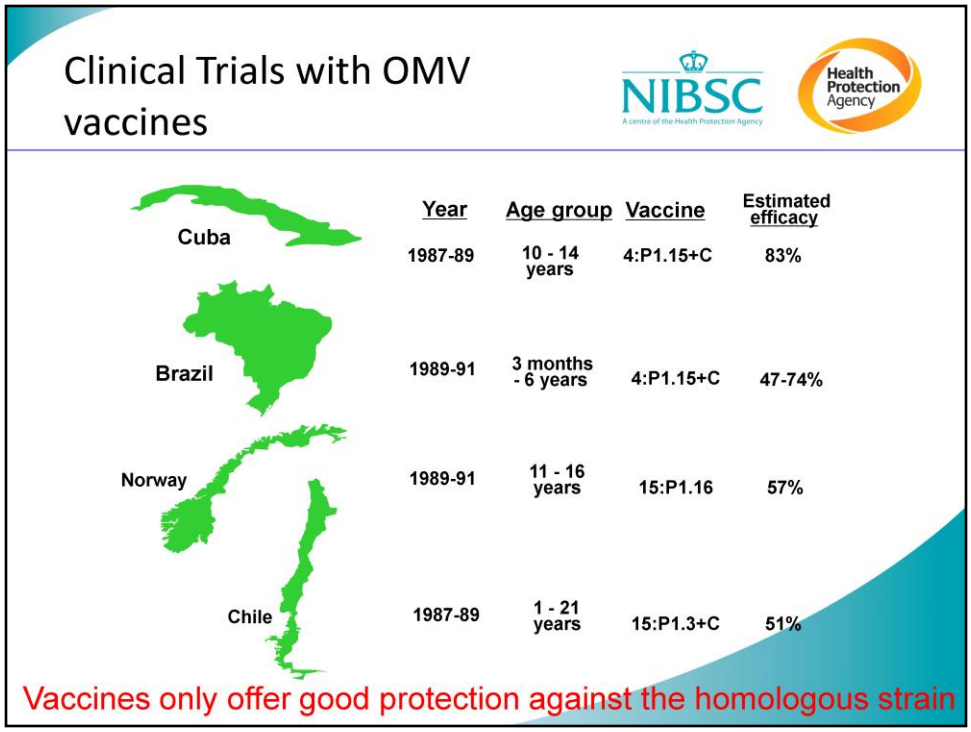


Meningococcal Outer Membrane Vesicle Vaccines

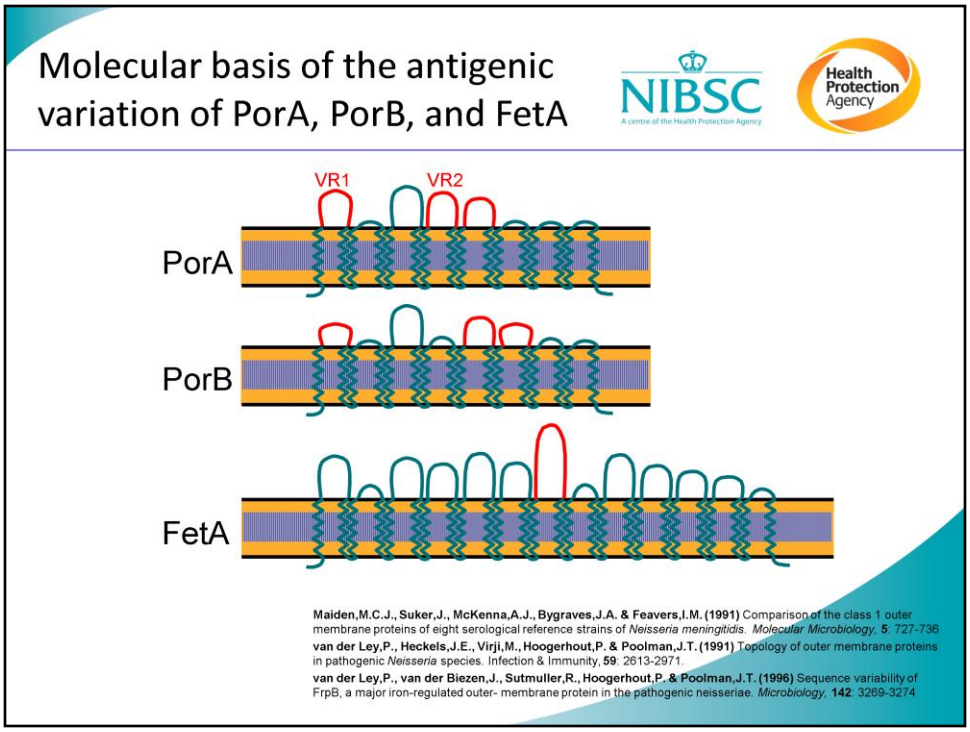
- OMVs are released spontaneously by meningococci *in vivo* and in culture
- They contain all the protein antigens usually associated with the OM
- Vaccines are produced by detergent extraction to reduce endotoxin content
- Hyper-producing mutants have been isolated



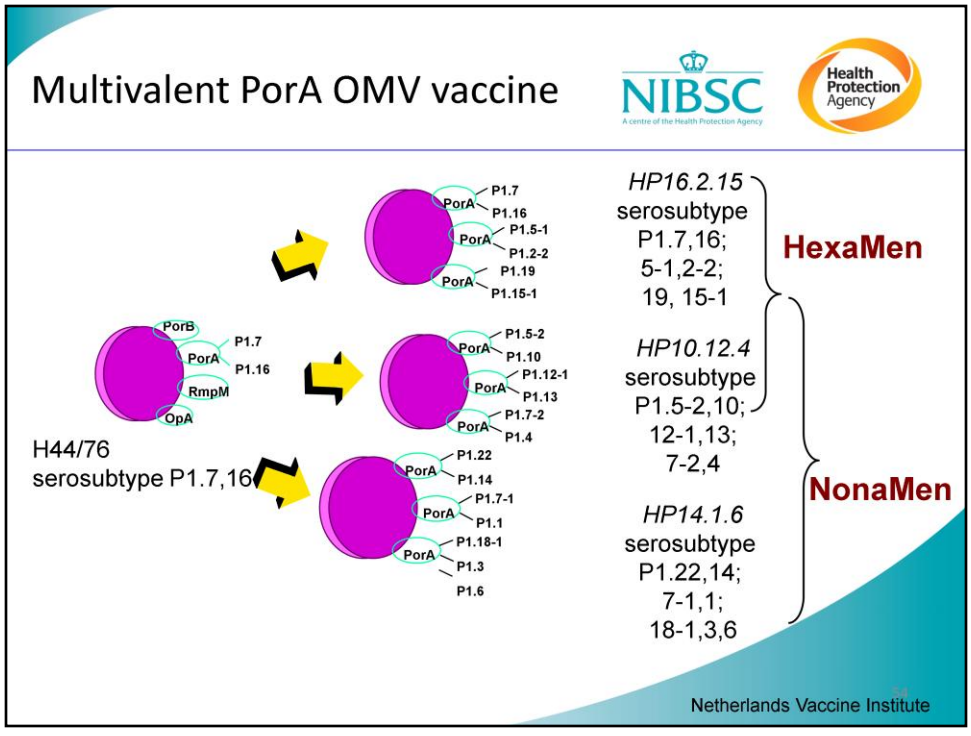
OMV size range 50-200 nm



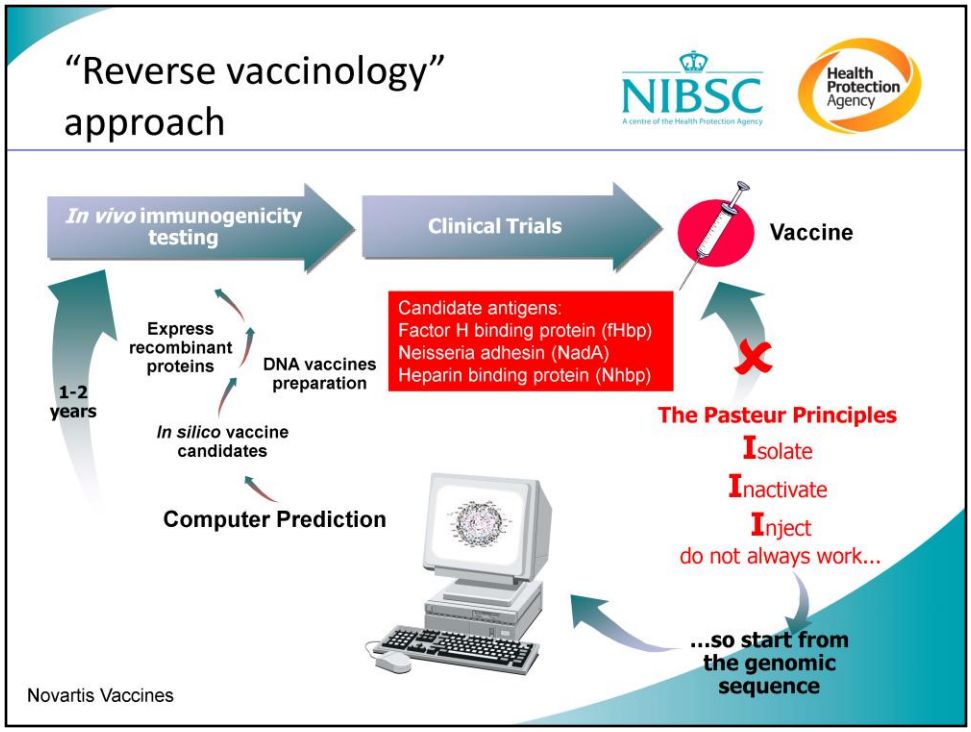
OMV vaccines have been evaluated in a number of efficacy studies. In general, protection is variable and strain specific, especially in the very young who are most at risk of infection. The predominant protective antibody response is directed at the PorA protein antigen i.e. PorA is said to be immunodominant in this vaccine. The vaccine is strain specific because PorA is antigenically variable; its amino acid sequence is different in different isolates.



Most of the variable amino acids in PorA reside in one of two cell-surface exposed loops known as VR1 and VR2. The protective immune response is mainly directed against these loops.

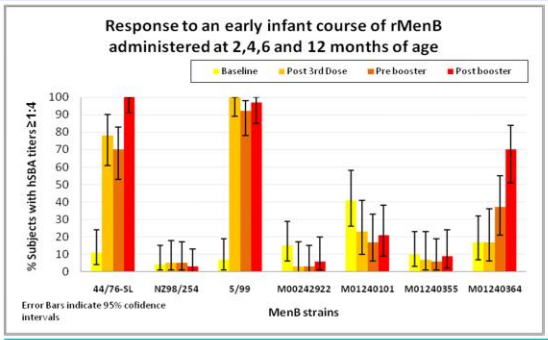


One solution to this problem is to develop an OMV vaccine that contains multiple PorA proteins. The NVI has done this by genetically engineering strains that each express three different PorAs. OMVs from these strains have been combined to make two vaccine formulations: Hexamen and Nonamen. The former has been evaluated in human trials; the latter has so far only been studied in mice.



In the era of genome sequence data, an alternative to the classical approach to identifying vaccine candidates is first to identify potential candidate antigens in the computer. The candidates are then over expressed in a suitable expression system. Novartis has used this method to identify three new meningococcal antigens. Sequence analysis indicates that these proteins are less variable than PorA and it is hoped that a vaccine based on these antigens would be broadly cross-protective against diverse meningococcal strains.

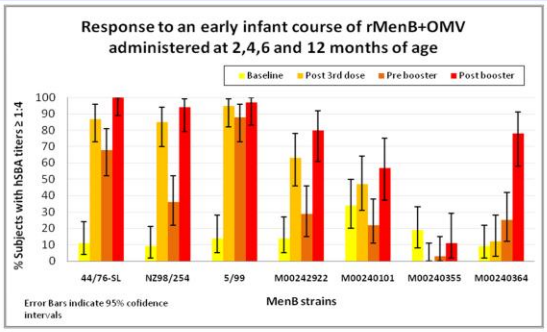
Clinical evaluation of Novartis vaccine formulations in young infants



Designation	ST	Clonal complex	Por A	fHBP	NadA variant	
44/76-SL	32	32	P1.7,16	1.1	-	Vaccine strains
NZ98/254	42	41/44	P1.7-2,4	1.10	-	
5/99	8	8	P1.5,2	2.8	2	
M00 242922	41	41/44	P1.7-2,4	1.4	-	Additional reference strains
M01 240101	1049	269	P1.19-1,15-11	1.11	-	
M01 240355	213	213	P1.22,14	3.4	5	
M01 240364	11	11	P1.5,2	3.4	2	

Snape *et al.* (2008) IPNC, Rotterdam
Su *et al.* (2009) ESPID, Brussels

Clinical evaluation of Novartis vaccine formulations in young infants



- Vaccine gives encouraging SBA results against the homologous strains
- No evidence of cross-reactive bactericidal response within fHbp variant subfamilies

Designation	ST	Clonal complex	Por A	fHBP	NadA variant	
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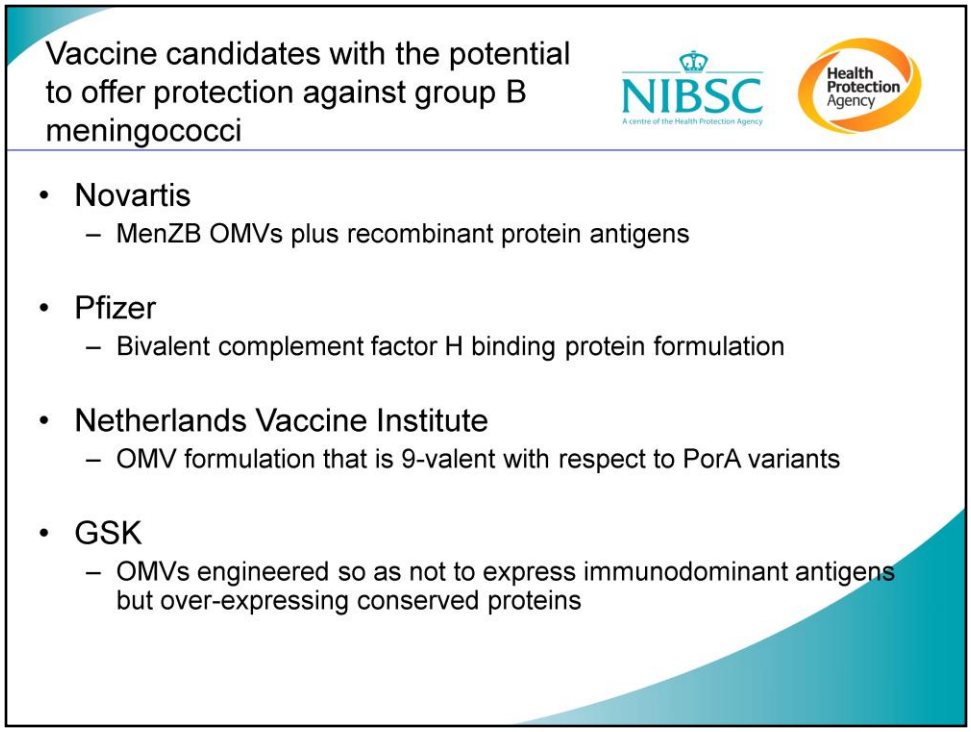

MenB vaccine summary.

In addition to the Novartis and NVI approaches, Pfizer is currently developing a bivalent fHbp vaccine and GSK is exploring the potential of making vesicles lacking immunodominant antigens. The rationale to this approach is that the immunodominant antigens are also the most variable (a consequence of immune-selection in the host); therefore, eliminating them from the vaccine should result in the more conserved antigens becoming more immunodominant and hence the vaccine would be more cross-protective.