Vaccines

Philip LaRussa
Division of Pediatric Infectious Diseases
Columbia University

• Historical Perspective
• Immunization Strategies
• Vaccine Safety
• Current Technology
• Routine Childhood & Adult Immunization Schedules
• Impact of Vaccines on Disease Burden
• Future Needs
• Background & Additional Information

Historical Perspective

• 1721, Lady Mary Montague
  – Observes variolation in Turkey & promotes its use in Europe
• 1774, Benjamin Jesty
  – Inoculates wife & 2 children with cowpox during a smallpox epidemic
  – Children are protected 15 years later after deliberate inoculation with smallpox
• 1796, Jenner
  – Milkmaids who had cowpox (vaccinia?) were immune to smallpox
  – Inoculated fluid from cowpox lesions into the skin of smallpox susceptible people (calf lymph-derived vaccinia virus)
  – “1st” use of a less virulent related species to protect against an exclusively human pathogen

• 1885: Louis Pasteur vaccinates Joseph Meister with rabies vaccine
  – Air-dried infected rabbit spinal cord:
    • started with avirulent virus, then proceeded with a series of more virulent strains
  – Coins “vaccination” in honor of Jenner
• 1955, Salk:
  – formalin-inactivated polio vaccine (IPV)
• 1962, Sabin:
  – Live attenuated polio vaccine (OPV, TOPV)
**Immunization Strategy**

- **Prevention of infection vs. symptoms**
- **Temporary vs. Long-lasting Immunity**
  - Passive protection: specific antibodies
    - Immediate Protection, but $t_{1/2} \approx 27$ days:
      - Antitoxins
      - Antibodies to Tetanus, Diphtheria, Botulinum toxins
    - Antisera to specific pathogens:
      - Hepatitis B, Varicella, Rabies, RSV
      - Pooled Human Immune Globulin: not specific
        - Immune Serum Globulin & Intravenous IG
  - Active: vaccination (Lag time, but long-lasting)
  - Active - Passive (HBIG+Hep B vac.; RIG+Rabies vac.)
- **Preventative (Polio) vs. Post-exposure (Rabies)**

**Target Populations for Immunization**

- **High Risk Groups Only (Rabies, Varicella in some countries)**
  - No effect on disease burden in general population
  - Vaccine must be highly effective
  - Must be able to reach all members of group
  - Less expensive in the short term
- **Universal Immunization (Polio, Rubella, Varicella in USA)**
  - Diminishes disease burden in general population
  - Pre-emptive immunization/ eventual high risk groups
  - Decreases risk of exposure
  - Planned access to target population
  - More cost-effective in long term
  - Requires extremely safe vaccines

**Immunization of High Risk Groups**

- **Travel**
  - Polio, Hepatitis A, Diphtheria, Japanese Encephalitis, Meningococcus, Yellow fever, Typhoid,
- **Occupation:**
  - Hepatitis B, Rabies, Anthrax, Plague, Rubella & Varicella
- **Age, illness, immunosuppression**
  - High risk for invasive pneumococcal disease:
    - Children $< 6$ years (Pneumococcal conjugate vaccine)
    - Elderly, high risk kids $\geq 6$ years (Pneumococcal polysaccharide vaccine)
  - Influenza: elderly, or cardiac or pulmonary disease
  - Severe varicella (live attenuated varicella vaccine):
    - leukemic children & HIV-infected kids with CD4 $\geq 25$
    - HIV-infected children [Inactivated polio vaccine]

**Administration**

- **Route**
  - Mimic route of natural infection: Oral polio vaccine, Live attenuated Intranasal Influenza vaccine
  - Parenteral (Intramuscular, subcutaneous)
- **Age at immunization**
  - Age distribution of natural infection:
    - In pre-vaccine era: $\geq 60\%$ of invasive H.influenzae type b infections occurred at $\leq 18$ months of age
  - Age-dependent immune response:
    - Polysaccharide antigens (HIB, Pneumo & Meningococcus) are poorly immunogenic at $\leq 2$ years of age
  - Ability to access population to be immunized:
    - Hepatitis B & rubella vaccines in infants vs. adolescents

**Immune Response to Immunization**

- **Protection vs. Sensitization**
- **Local vs. Systemic immunity:**
  - Mucosal surfaces (gut, respiratory, genital-urinary tracts, eye) vs. intravascular space
- **Antibody Response:**
  - T-cell dependent & T-cell independent antigens stimulate naive B cells to secrete epitope specific antibodies:
    - Prevent attachment to receptors
    - Inactivate toxins
    - Neutralize live viruses
    - Opsonization
- **Cell-mediated Response:**
  - T-cell response → maturation of naive to mature cytotoxic T cells → lyse infected host cells displaying pathogen-specific antigens on their surface in the context of MHC-I molecules

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Immune Response to Immunization

• Primary response
  – 1st exposure to the antigen
  – 7-10 day lag time between exposure and production of antibody and cell-mediated responses
  – Initial antibody response is IgM, later switch to IgG
  – Establish populations of memory T & B cells

• Secondary response
  – Repeat exposure to the antigen (or to the pathogen)
  – Shortened lag time between exposure and production of antibody and cell-mediated responses
  – Antibody response is almost all IgG
  – Rapid expansion/ Memory T & B cell populations

Current Technology

• Inactivated whole organism:
  – Whole cell Pertussis, eIPV, Hepatitis A, Rabies, Influenza(disrupted), plasma-derived Hepatitis B (no longer available in US)

• Live organism from a related or different species:
  – Vaccinia, Bacille Calmette-Guerin (BCG, also attenuated by serial passage)

• Live attenuated organism:
  – Oral Polio, Measles, Mumps, Rubella, Varicella, Cold-adapted Influenza, Yellow fever
  – Attenuated by passage in tissue culture

• Toxoids: inactivated Diphtheria, Tetanus toxins

• Combination Vaccines:

Current Technology

• Specific subunit/antigen(s), extracted and purified:
  – Acellular Pertussis Vaccines:
    • PT (Pertussis toxoid), FHA (filamentous hemagglutinin), Pertactin, Agglutinogens
  – Polysaccharides (T-cell independent antigens):
    • Hæmophilus(no longer available), Meningococcus, Pneumococcus
    – Influenza surface glycoproteins (HA, NA)
  – Conjugated antigens (T-cell dependent):
    • HiB: PRP-D, PRP-T, PRP-GMP, HBoC(crm197)
    • Pneumococcal Conjugate:
      – CRM 4V: 4, 6B, 9V, 14, 19F, 23F, 18C
    • Meningococcus A, C, W-135 & Y conjugated to diphtheria toxoid

Current Technology

• Recombinant antigens: HBsAg/ yeast

• Virus-like particles:
  – Major capsid proteins of human papillomavirus serotypes 6, 11, 16 & 18 expressed in eucaryotic cells
  – Quadrivalent Vaccine efficacy:
    • 99-100% vs HPV 16/18 related Cervical Intraepithelial neoplasia (CIN) 2/3 in uninfected women
    • 27% efficacy in women who are recently infected
    • No efficacy in those with established infection
    • To be licensed for use in females 9-26 years in 2006
      – Males and a bivalent 16/18 vaccine later on
      – Younger age groups to follow

Establishing Causal Link: Adverse Event and Vaccine

<table>
<thead>
<tr>
<th>Unique lab result</th>
<th>Illness or Syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unique clinical syndrome</td>
<td>Yes</td>
</tr>
<tr>
<td>Epidemiologic study (VMERS = Blinded cell “a”)</td>
<td>Vaccination</td>
</tr>
<tr>
<td>Rate in vaccinated = a/c</td>
<td>Rate in unvaccinated = d/b</td>
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Current Technology

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Adjuvants

- Non-pathogen related additives that improve immunogenicity
- Aluminum salts are most common
  - Hepatitis b vaccine, tetanus and diphtheria toxoids
- Mechanisms of action?
  - Formation of an antigen depot at the inoculation site
    - Water/oil emulsions & alum
  - Mobilization of T cell response:
    - Protein carriers, polyA/polyU
  - Up-regulation of Ig receptors on B cells:
    - B-cell mitogens, antigen polymerizing agents
  - Increased uptake by Antigen-presenting cells:
    - MDP (muramyl dipeptide) derivatives, LPS, Lipid A
  - Cytokine induction & secretion

Routine Adult Immunizations

- Diphtheria & Tetanus boosters every 10 years
  - Pertussis may be added to the adolescent & adult schedule
- Influenza A/B
  - Yearly if > 55 years or high risk
  - Eventually: all adults regardless of age
- Pneumococcal polysaccharide (23-valent)
  - High risk adults
  - ≥ 65 years
  - Future use of an “adult” conjugate vaccine???
- Hepatitis B: if high risk
  - If not immune:
    - Varicella, Rubella
    - Measles & Mumps: if born after 1956

Most Pressing Future Needs

- HIV
- Malaria
- Tuberculosis:
  - Improved BCG vaccine: rBCG30
    - Contains an extra copy of the major secretory protein (Ag85b) → improved immunogenicity & protection in animal models
    - Phase I clinical trials in humans completed
  - Prime-Boost strategy:
    - Prime with BCG
    - Boost with MVA (Modified Vaccinia Ankara) vector containing the gene for TB antigen 85A
    - More robust CD4 response than either vaccine alone

Malaria, still on the horizon?

- Unique Challenge for Immunization:
  - Multiple species:
    - P. falciparum most important
    - also P. vivax, ovale, malarie
  - Multiple life cycle stages:
    - Sporozoites, (liver-stage schizonts), merozoites, blood stages, gametocytes
  - Antigens are polymorphic and/ or undergo clonal variation
  - Constant exposure to the pathogen:
    - “natural immunity” – chronic low-grade infection with constant exposure to changing antigens
**Malaria, still on the horizon?**

- Approaches to vaccine development
  - Irradiated sporozoite vaccine = “gold standard”
  - Stage specific recombinant antigens:
    - Circumsporozoite proteins (CSP):
      - RTS.S: segment of tandem-repeat region of CSP + flanking T cell epitopes + hepatitis b surface antigen expressed in yeast + 3-component adjuvant
    - Merozoite surface protein 1 (MSP1,)
    - RBC schizont antigen (SERA)
    - Gametocyte antigens (Pf625)
    - Multiple Antigen Peptides (MAPs)
    - Strong adjuvants

- Inadequate Long-term protection:
  - Failure to induce adequate memory T-cell responses?
  - Will prime-boost strategies work better?

- Additional references:
  - Targett, Trends in Parasitology, 2005
  - Okie, NEJM, 2005

**Historical Perspective**

- 1886, Salmon/ Smith: killed hog cholera “virus” vaccine (salmonella)
  - led to killed vaccines for typhoid, cholera & plague
- 1909, Smith: inactivated diphtheria toxin (toxoid) protects guinea pigs
  - led to diphtheria & tetanus toxoid vaccines for humans
- 1927, Calmette & Guerin: BCG
  - attenuated by passage in beef bile over 13 years of Mycobacterium bovis
- 1931, Goodpasture: chorioallantoic membrane/hen’s egg
  - safe, reliable method for growing viruses for vaccines
- 1937, Live attenuated yellow fever vaccine
  - passage in mouse brain & chorioallantoic membrane/hen’s egg (17D strain)
- 1955, Salk: formalin-inactivated polio vaccine (IPV)
- 1962, Sabin: Live attenuated polio vaccine (OPV, TOPV)

**Vaccination Against Smallpox: Vaccinia virus**

JAMA, June 9, 1999-vol.281(22):2127-37

**Current Technology**

- Recombinant L-OspA Lyme vaccine:
  - No longer available
  - E. coli transformed with plasmid containing OspA gene
  - Lipid moiety added after translation
  - 30 ug of purified antigen adsorbed to aluminum hydroxide
  - Production of antibody to spirochete outer surface lipoprotein expressed in the tick phase
  - Antibody-mediated killing in the tick

**Additional Background Slides**
Inactivated Influenza Vaccines

- **Current Technology:**
  - Live reassortant viruses consisting of high growth virus and vaccine candidates containing the selected hemagglutinins and neuraminidase components which are then grown on embryonated chick eggs.
  - Vaccine viruses are then inactivated and detergent-disrupted.

- **Components of the 2005-6 vaccine:**
  - A/California/7/2004 (H3N2)-like
  - A/New Caledonia/20/99 (H1N1)-like
  - B/Shanghai/361/2002
  - Selected because of growth properties and because they are representative of strains likely to circulate in the US during the 2005–06 season.
  - Since Influenza A (H1N2) viruses are a reassortant of A(H1N1) & (H3N2) viruses, antibodies directed against A (H1N1) and A (H3N2) vaccine strains provides protection against circulating A (H1N2).

Future Influenza Vaccines

- **Example of Reverse Genetics Technique for production of Inactivated Influenza Vaccines:**
  - Extract RNA from master vaccine strain (H1N1) & candidate wild-type strains (e.g. H5N2, H7N2, H5N1, H5N8)
  - Amplify (RT-PCR) genes for HA & NA from wild-type strains & “backbone” genes from master vaccine strain (Polymerase complex genes, etc.)
  - Clone each into plasmids & transfect 293T cells
  - Collect reassortant viruses (rH5N1,…containing HA & NA genes from wild-type strains & backbone genes from master vaccine strain)
  - Infect ECE (embryonated chick eggs) or immortalized cell lines like Marcus Darby Canine kidney cells (MDCK)
  - Disrupt cells, collect, inactivate vaccine virus
  - Can modify this technique for cold-adapted live attenuated vaccines

Selected References:
- Lee, et. al. Vaccine, 2004
- Webby, et. al. Lancet, 2004
- Nicolson, et. al. Lancet, 2005

Rotavirus Vaccine

- **RotaTeq Vaccine Study:**
  - Pentavalent bovine-human reassortant vaccine
  - VP7 genes of serotypes G1, G2, G3, G4 and P-type P1A
  - 70,000 placebo-controlled study:
    - 70% efficacy vs. any vaccine-serotype-related disease
    - 98% vs. severe disease
    - 85, 94, 96% ↓ in office visits, ED & hospitalizations
    - Intussusception:
      - 6 & 5 cases in the overall vaccine & placebo groups
      - 0 & 1 in vaccine & placebo groups after the 1st dose

Down the Road

- **Viral Vectors:**
  - Vaccinia:
    - good cytotoxic T-cell response (CTL)
    - pre-existing immunity to vaccinia limits use
    - primary response to vector limits response to booster doses of vectored vaccine
    - Occasionally, poor responses to inserted antigens
    - Canarypox, Adenovirus, Baculovirus
    - Varicella-Hepatitis B

On the Horizon

- **New Combination Vaccines:**
  - Tdap (Tetanus-Low dose diphtheria-acellular pertussis)

- **Maternal Immunization/neonatal disease**
  - Tetanus
  - Group B Streptococcus:
    - Capsular Polysaccharides (Ia, Ib, II, III, IV) conjugated to tetanus toxoid
    - “Universal” surface protein(s) vaccine covering all serotypes?
  - Live attenuated Dengue type 1-4 vaccines
  - New live attenuated rotavirus vaccine

- **Replicons:**
  - RNA viruses engineered to consist of a virus coat housing a genome with structural genes replaced by gene for the immunizing antigen:
    - Infection of host cell
    - Large quantities of mRNA for the desired antigen
    - No replication of parent virus (no structural genes)
• Bacterial mutants as vectors or attenuated vaccines
  – BCG, Salmonella, Shigella, Listeria
  • Auxotrophic mutant Shigella:
    – invasion of target cell but can’t replicate without a key nutrient
    – dies, releasing episomal plasmid DNA coding for desired antigen
  • Auxotrophic mutant BCG & M. tuberculosis (MTB)
    – defect in purine synthesis pathway → unable to replicate in & lyse macrophages
    – immunized guinea pigs protected after challenge with virulent MTB
  • Salmonella auxotrophs expressing IL-2
    – protection of immunized mice after intraperitoneal challenge ←
      Nitric oxide & IFN-γ production by peritoneal cells

• Peptides:
  – As the Immunogen:
    • B-cell epitopes:
      – Conserved
      – B cells usually respond to 3D shape of the epitope
    • T-cell epitope:
      – MHC-restricted: Multiple epitopes for major haplotypes?
      – T cell epitopes are usually linear sequences of aa’s
  – As the Carrier: should elicit T-cell help

• Potential adjuvants under evaluation:
  • Monophosphoryl lipid A
  • MF59 (emulsion of oil & surfactants)
  • SAF-1 (oil based emulsion of MDP + non-ionic block copolymers)
  • Saponin derivatives
  • Polymers (polyphosphazene)
  • Bacterial toxins (cholera & E. coli HL)
  – Orally cholera toxin → Th2 response → IgG1, IgE, mucosal IgA
  • Cytokines:
    – IL-4 → mucosal IgA & IgG
    – IL-4 → type 2 T-cell response (Th2/Tc2) → potent Ig production
    – IL-12 → type 1 T-cell response (Th1/Tc1) → potent γ-IFN & cytotoxic T-cell responses

• Delivery Systems:
  – Liposomes & Microcapsules
  • Polymers surrounding antigens
  • PLGA (disposable suture material)
  • Potential uses:
    – Prolonged degradation ⇒ fewer doses for primary immunization
    – Oral vaccines: protection from stomach acidity & selective uptake by M cells in Peyer’s patches

• Nasal & Oral Vaccines
  – Mucosal routes → mucosal immune responses
  – Respiratory & enteric pathogens
  – Examples:
    • Oral cholera vaccines:
      – Cholera toxin B subunit/ Inactivated whole cell(B-WC)
      – Live attenuated deletion-mutant strains
      – Bivalent(O1/O139) B subunit/Inactivated whole cell
    • Oral vaccines for enterotoxigenic E. coli
      – Antibody to Cholera toxin B subunit cross-reacts with E. coli LT-B (heat labile toxin)

• Edible Plant Vaccines:
  – Transgenic plants expressing protein antigens:
    • Phase I/II trials of transgenic potatoes expressing the binding subunit of cholera toxin: safe & immunogenic
    • Phase I/II trials of transgenic potatoes expressing HBsAg as a booster after traditional vaccine
  – Infection of edible plants with chimeric plant viruses expressing the antigen of choice on its surface
  – Effect of cooking on immunogenicity in humans?
**Down the Road**

- **Nucleic Acid Vaccines (Naked DNA):**
  - Bacterial plasmids carrying:
    - Genes encoding immunizing antigen or replication-defective viral vectors
    - Strong viral promoter
  - Intramuscular injection
  - Generate MHC-I restricted CTL responses
  - Antigen is produced in mammalian cells:
    - More appropriate antigen conformation

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**Key for Vaccine Abbreviations**

- BCG: Bacille Calmette-Guérin vaccine
- CRM197: nontoxic mutant diphtheria toxin
- DTaP: Diphtheria, Tetanus, Pertussis (acellular)
- DTP: Diphtheria, Tetanus, Pertussis (whole cell)
- HibOC: a Hib vaccine that uses CRM197 as a carrier protein conjugated to PRP
- Hep A, Hep B: Hepatitis A or B vaccine
- Hib: Haemophilus influenzae, type b
- IPV/eIPV: Inactivated polio vaccine or enhanced potency IPV
- MMR: Measles, Mumps, Rubella vaccine
- MMRV: Measles, Mumps, Rubella, Varicella vaccine
- OMP: outer membrane protein of Neisseria meningitides
- OPV or eOPV: live or attenuated oral polio vaccine
- OspA: outer surface protein A of Lyme spirochete
- Polio: refers to either OPV or eOPV
- PRP: polyribosylribitol phosphate (the capsular polysaccharide of Hib)
- PRP-T, PRP-D, PRP-OMP: Hib vaccines with the PRP conjugated to T(tetanus), D(diphtheria) or OMP, respectively as the carrier protein
- Var: varicella vaccine