Vaccines

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• 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

• “Ancient Times”, the Baluchi people
  – Encouraged children with wounds on their hands to touch skin lesions of cow/camelpox

• “Centuries ago” Variolation in India, China?
  – inoculation of fluid or scabs from smallpox lesions into skin or intranasally of susceptibles
  – usually mild illness, occasionally severe disease with spread to others

• 11th century/Iran
  – applied dried liver/rabid dog on wound of bitten person

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
“The Cow Pock: The Wonderful Effects of the New Inoculation!”
James Gillnay, 1802 vide the publication of the Anti Vaccine Society

**Historical Perspective**

- **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 ≥ 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 ≥ 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: ≥ 60% of invasive H.influenzae type b infections occurred at ≤ 18 months of age
  – Age-dependent immune response:
    • Polysaccharide antigens (HIB, Pneumo & Meningococcus) are poorly immunogenic at ≤ 2 years of age
  – Ability to access population to be immunized:
    • Hepatitis B & rubella vaccines in infants vs. adolescents
Administration

• Type of Antigen & Number of Doses needed:
  • Availability of Live vs. killed vaccine
  • Likelihood of Take vs. No Take with 1st dose
  • Waning immunity after 1st dose
  • T-cell dependent vs. -independent response
• Safety concerns:
  – ability of host to control replication of live attenuated vaccine strains

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(Th2) & T-cell independent antigens stimulate naïve B cells to secrete epitope specific antibodies:
    • Prevent attachment to receptors
    • Inactivate toxins
    • Neutralize live viruses
    • Opsonization
• Cell-mediated Response:
  – Th1 response → maturation of naïve to mature cytotoxic T cells → lyse infected host cells displaying pathogen-specific antigens on their surface in the context of MHC-I molecules
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
Establishing Causal Link: Adverse Event and Vaccine

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<th>Unique lab result</th>
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<table>
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<tr>
<th>Unique clinical syndrome</th>
<th>Epidemiologic study</th>
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<td>(VAERS = biased cell “a”)</td>
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<th>Vaccination</th>
<th>Yes</th>
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<tr>
<td></td>
<td>a</td>
<td>b</td>
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<td>c</td>
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Rate in vaccinated = a/(a+b)
Rate in unvaccinated = c/(c+d)

For rare events: consider case-control design study

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-OMP, HBoC\(\text{crm197}\)
  - Pneumococcal Conjugate
    - CRM 197- 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
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 Th 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 (MSP1α)
    • RBC schizont antigen (SERA)
    • Gametocyte antigens (Pfs25)
  – Multiple Antigen Peptides (MAPs)
  – Strong adjuvants

Malaria, still on the horizon?

• 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
Additional Background Slides

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)
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
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 & transfet 293T cells
  • Collect reassortant viruses (H5N1,…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
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

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

Down the Road

• 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)
Down the Road

• 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

Down the Road

• 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
Down the Road

• 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-6 → mucosal IgA & IgG
    – IL-4 → type 2 T-cell response (Th2/Th2) → potent Ig production
    – IL-12 → type 1 T-cell response (Th1/Th1) → potent γ-IFN & cytotoxic T-cell responses

Down the Road

• 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
Down the Road

• 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)

Down the Road

• 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 MCH-I restricted CTL responses
  – Antigen is produced in mammalian cells:
    • More appropriate antigen conformation

Key for Vaccine Abbreviations

• BCG: Bacille Calmette-Guérin vaccine
• CRM<sub>197</sub>: nontoxic mutant diphtheria toxin
• DTaP: Diphtheria, Tetanus, Pertussis (acellular)
• DTP: Diphtheria, Tetanus, Pertussis (whole cell)
• HbOC: a HIB vaccine that uses CRM<sub>197</sub> as a carrier protein conjugated to PRP
• Hep A, Hep B: hepatitis A or B vaccine
• Hib: Hemophilus 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 meningitidis
• OPV or OTPV: live attenuated oral(trivalent) polio vaccine
• OspA: outer surface protein A of lyme spirochete
• Polio: refers to either OPV or eIPV
• PRP: polyribosilribotol 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