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

Historical Perspective: Louis Pasteur

- 1879: “weakened” chicken cholera culture (Pasteurella multica) by exposure to air
  - Immunity after challenge with virulent organisms
- 1881: 2 doses of heated anthrax bacilli
  - All vaccinated animals were protected (21 sheep, 6 cattle & a goat) after challenge with virulent organisms
  - Most unvaccinated, challenged control animals died (23 sheep & 1 goat, 4 cows became ill)
- 1885: Vaccinates Joseph Meister with rabies vaccine
  - Air-dried infected rabbit spinal cord:
    - Stated with avirulent virus, then proceeded with a series of more virulent strains
- Coins “vaccination” in honor of Jenner
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)

Immunization Strategy

• Prevention of infection vs. symptoms
• Temporary vs. Long-lasting Immunity
  – Passive (Immediate Protection, but t1/2 ≈ 27 days):
    • Antitoxins
      – Tetanus[human, equine], Diphtheria[equine], Botulinum[human, equine]
    • Antisera to specific pathogens:
      – Hepatitis B, Varicella, Rabies, RSV
    • Pooled Humane Immune Globulin:
      – Immune Serum Globulin & Intravenous IG
  – Active (Lag time, but long-lasting)
    – Active - Passive (HBIG+Hep B vac.; RIG+Rabies vac.)
• Preventative vs. Post-exposure (Rabies)

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
  – Pneumococcal Conjugate: high risk < 6 years
  – Pneumococcal Polysaccharide/elderly, high risk ≥ 6 years
  – Influenza: elderly, cardiac or pulmonary disease
  – Varicella: leukemic children & others ?, elderly for zoster
  – Inactivated polio: HIV

Target Populations for Immunization

• High Risk Groups
  – No effect on disease 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:
  – Diminishes disease 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

Administration

• Route
  – Mimic route of natural infection?
  – 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

• Number of Doses needed
  – Type of Antigen
    • Live vs. killed
    • T-cell dependent vs. -independent
    • Safety concerns: ability of host to control replication of live attenuated vaccine strains
  – One vs. Multiple doses:
    • Take vs. No Take
    • Booster Response
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) & independent antigens stimulate naïve B cells to secrete epitope specific antibodies:
    - Prevent attachment to receptors
    - Neutralization
    - Opsonization
- Cell-mediated Response:
  - T-cell response → maturation of naïve to mature cytotoxic T cells → lyse infected host cells that display pathogen-specific antigens on their surface in the context of MHC-I molecules

- 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

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Current Technology

- Inactivated whole organism:
  - Whole cell Pertussis, eIPV, Hepatitis A, Rabies, Influenza (disrupted), Hepatitis B (no longer available in US)
- Live organism/ 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
- Toxoids: Diphtheria, Tetanus
- Combination Vaccines:

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Establishing Causal Link: Adverse Event and Vaccine

- Unique lab result
- Illness or Syndrome
- Unique clinical syndrome
- Vaccination

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Current Technology

- Specific subunit/antigen(s), extracted and purified:
  - Acellular Pertussis Vaccines:
    - PT (Pertussis toxoid), FHA (filamentous hemagglutinin), Pertactin, Agglutinogens
  - Polisaccarides (T-cell independent antigens):
    - Haemophilus (no longer available), Meningococcus, Pneumococcus
  - Influenza surface glycoproteins (HA, NA)
- Conjugated antigens (T-cell dependent):
  - Hib: PRP-D, PRP-T, PRP-OMP, HibOc (crm197)
  - Pneumococcal Conjugate:
    - CRM 197- 4, 6B, 9V, 14, 19F, 23F, 18C
  - Meningococcus A & C
- Recombinant antigens: HBsAg/ yeast
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 polymeric 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
- Influenza A/B
  - Yearly if > 65 years or high risk
  - Eventually: all adults regardless of age
- Pneumococcal polysaccharide (23-valent)
  - Every 5 years if > 65
  - Future use of an “adult” conjugate vaccine???
- Hepatitis B: if high risk
- If not immune:
  - Varicella
  - Measles & Mumps: if born after 1956
  - Rubella

On the Horizon

- New Combination Vaccines:
  - MMRV
- Maternal Immunization/neonatal disease
  - Tetanus
  - Group B Streptococcus (protein conjugate)
- Live attenuated Dengue type 1-4 vaccines
- New live attenuated rotavirus vaccine

Malaria, On the Horizon?

- Unique Challenge for Immunization:
  - Multiple species (P. falciparum most important)
  - Multiple life cycle stages
  - Constant exposure to the pathogen
- Approaches to vaccine development
  - Stage specific recombinant antigens:
    - Circumsporozoite protein (CS)
    - Merozoite surface protein 1 (MSP1)
    - RBC schizont antigen (SERA)
    - Gametocyte antigens (Pf625)
  - Multiple Antigen Peptides (MAPs)
  - Strong adjuvants
- Inconsistent/short-lived protection
Future Needs

- HIV
- Malaria
- Tuberculosis (more effective than BCG)

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

- 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
  - Virus-like particles: Recombinant L1
    - major capsid protein of human papillomavirus expressed in eucaryotic 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
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 co-polymers)
  • Saponin derivatives
  • Polymers (polyphosphazene)
  • Bacterial toxins (cholera & E.coli HL)
    – Orally cholera toxin → Th2 response → IgG1, IgE, mucosal IgA
  • Cytokines:
    – IL-6 → mucosal IgA & IgE
    – IL-4 → type 2 T-cell response (Th2): potent Ig production
    – IL-12 → type 1 T-cell response (Th1): 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?

• 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

Vaccination Against Smallpox: Vaccinia virus

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

• **Cross-species reassortment virus:** Tetravalent Rotavirus (Rv): **No longer available**
  – Live-attenuated, orally administered
  – Derived from four group A Rvs
  – 3 are single gene reassortments of the VP7 gene of human pathogen origin (types G1, G2, and G4):
    • Each contains the parent human pathogen gene encoding the G protein & 10 genes from the parent rhesus Rvs
  – 4th strain: rhesus Rv type G3 ~ human G3
  – Removed from immunization schedule ⇐ ↑ risk of intussusception, especially after 1st dose

Current Technology

• **Recombinant L-OspA Lyme vaccine:**
  – **No longer available**
  – E. coli transformed with plasmid containing OspA gene
  – Lipid moiety added after translation
  – 30 μg 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

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)
• **HBOC:** a Hib vaccine that uses CRM197 as a carrier protein conjugated to PRP
• **Hep A, Hep B:** Hepatitis A or B vaccine
• **Hib:** Hæmophilus influenzae type b
• **IPV:** Inactivated polio vaccine
• **OPV:** Oral polio vaccine
• **OMP:** Outer membrane protein of Neisseria meningitidis
• **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
• **Polio:** refers to either OPV or eIPV
• **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