

Vaccines

Philip LaRussa
Division of Pediatric Infectious Diseases
Columbia University

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



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

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

- **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)**

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 ≤ 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

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

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

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 $CD4 \geq 25\%$
 - HIV-infected children (Inactivated polio vaccine)

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 \rightarrow maturation of naïve to mature cytotoxic T cells \rightarrow 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

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:**
 - DTP, MMRV, DTP-HIB, HIB-Hep.B, DTaP- Hep.B-IPV

Evolution of Immunization Program and Prominence of Vaccine Safety



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):
 - Haemophilus(no longer available), Meningococcus, Pneumococcus
 - Influenza surface glycoproteins (HA, NA)
- **Conjugated antigens (T-cell dependent):**
 - HiB: PRP-D, PRP-T, PRP-OMP, HBoC(czm197)
 - Pneumococcal Conjugate
 - CRM 197- 4, 6B, 9V, 14, 19F, 23F, 18C
 - Meningococcus A, C, W-135 & Y conjugated to diphtheria toxoid

Establishing Causal Link: Adverse Event and Vaccine

Unique lab result	Illness or Syndrome	
	Yes	No
Unique clinical syndrome	a	b
Epidemiologic study (VAERS = biased cell "a")	c	d

Vaccination: Yes (rows a, c), No (rows b, d)
 Rate in vaccinated = $a/a+b$
 Rate in unvaccinated = $c/c+d$

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**

Comparison of Maximum and Current Reported Morbidity, Vaccine-Preventable Diseases and Vaccine Adverse Events, United States

Disease	Pre-Vaccine Era*	Year	1997**	Percentage Change
Diphtheria	206,939	(1921)	5	-99.99
Measles	894,134	(1941)	135	-99.98
Mumps	152,209	(1968)	612	-99.60
Pertussis	265,269	(1934)	5,519	-97.92
Polio (wild)	21,269	(1952)	0	-100.00
Rubella	57,688	(1969)	151	-96.72
Cong. Rubella Synd.	20,000*	(1964-9)	4	-99.98
Tetanus	1,560*	(1948)	43	-97.24
Invasive Hib Disease	20,000*	(1984)	165	-99.18
Total	1,639,066		6,644	-99.59

Vaccine Adverse Events 0 11,365 +++

* Maximum cases reported in pre-vaccine era, 5-yr year
 ** 1997: maximum for national reporting available, 1/1/96 to present era
 *** 100,000/yr

Recommended Childhood and Adolescent Immunization Schedule UNITED STATES • 2005

Vaccine	Age	Birth	1 month	2 months	4 months	6 months	12 months	15 months	18 months	24 months	4-6 years	11-12 years	13-18 years
Hepatitis B		HepB #1		HepB #2			HepB #3					HepB Series	
Diphtheria, Tetanus, Pertussis ¹			DTaP	DTaP	DTaP		DTaP				DTaP	Td	Td
Haemophilus influenzae type b ¹			Hb	Hb	Hb		Hb						
Inactivated Poliovirus			IPV	IPV			IPV				IPV		
Measles, Mumps, Rubella ¹							MMR #1				MMR #2	MMR #2	
Varicella ¹							Varicella					Varicella	
Pneumococcal Conjugate ¹			PCV	PCV	PCV		PCV				PCV	PPV	
Influenza ²							Influenza (Yearly)				Influenza (Yearly)		
Hepatitis A ¹												Hepatitis A Series	

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 (MSP1₁₉)
 - RBC schizont antigen (SERA)
 - Gametocyte antigens (Pfs25)
 - Multiple Antigen Peptides (MAPs)
 - Strong adjuvants

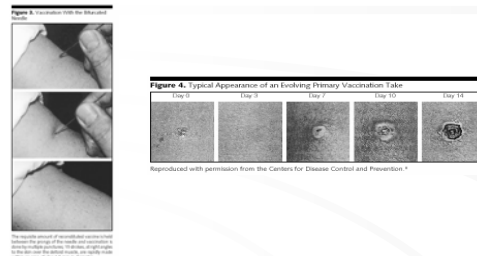
Historical Perspective

- **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)

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
 - http://www.who.int/vaccine_research/documents/en/malaria_table.pdf

Vaccination Against Smallpox : Vaccinia virus



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

Additional Background Slides

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)

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

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
- QuickTime™ and a TIFF (Uncompressed) decompressor are needed to see this picture.
- Can substitute MDCK cells →

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**

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

• Delivery Systems:

- Liposomes & Microcapsules
 - Polymers surrounding antigens
 - PLGA (disposable suture material)
- Potential uses:
 - Prolonged degradation \Rightarrow fewer doses for primary immunization
 - Oral vaccines: protection from stomach acidity & selective uptake by M cells in Peyer's patches

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

• 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

• 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/ Tc2) → potent Ig production
 - IL-12 → type 1 T-cell response (Th1/ Tc1) → potent γ -IFN & cytotoxic T-cell responses

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
- 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: *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 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: polyribosilribitol 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