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 pneumoccocal 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:
    - Polysaccaride 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

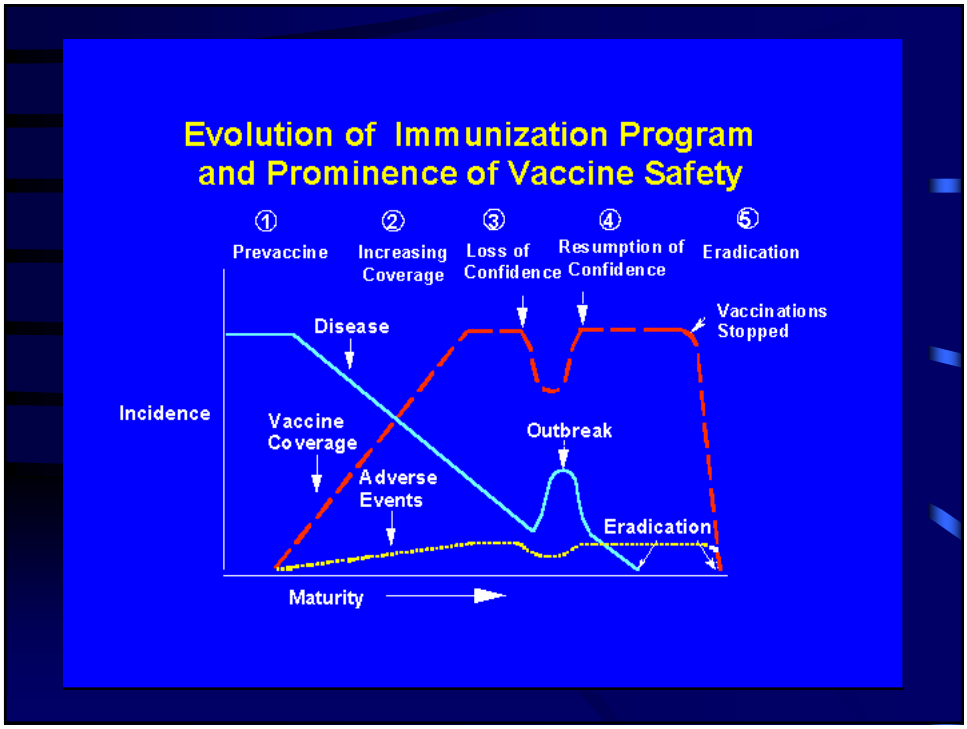
- Type of Antigen & Number of Doses needed:
  - Availability of Live vs. killed vaccine
  - Likelihood of Take vs. No Take with 1st dose
  - Waning immunity after 1st dose
  - T-cell dependent vs. -independent response
  - Safety concerns:
    - ability of host to control replication of live attenuated 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(T_{h2}) & 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:
  - T_{h1} 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**



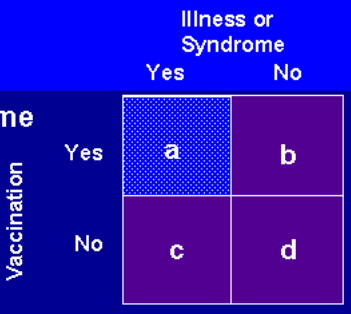
Establishing Causal Link: Adverse Event and Vaccine

Unique lab result

Unique clinical syndrome

Epidemiologic study

(VAERS = biased cell "a")



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

Current Technology

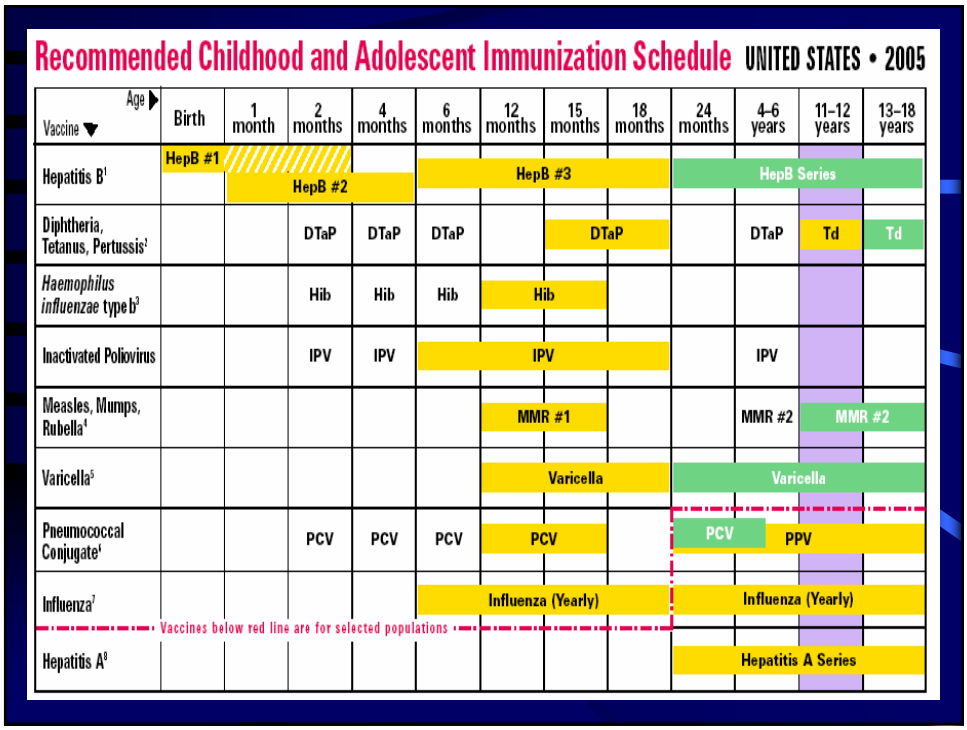
- **Specific subunit/antigen(s), extracted and purified:**
  - Acellular Pertussis Vaccines:
    - PT (Pertussis toxoid), FHA (filamentous hemagglutinin), Pertactin, Agglutinogens
  - Polysaccarides (T-cell independent antigens):
    - Hæmophilus(no longer available), Meningococcus, Pneumococcus
  - Influenza surface glycoproteins (HA, NA)
- **Conjugated antigens (T-cell dependent):**
  - HiB: PRP-D, PRP-T, PRP-OMP, HBoC(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**



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[1]	HepB #1	HepB #2			HepB #3				HepB Series			
Diphtheria, Tetanus, Pertussis[2]			DTaP	DTaP	DTaP		DTaP			DTaP	Td	Td
Haemophilus influenzae type b[3]			Hib	Hib	Hib	Hib						
Inactivated Poliovirus			IPV	IPV	IPV					IPV		
Measles, Mumps, Rubella[4]						MMR #1				MMR #2	MMR #2	
Varicella[5]						Varicella			Varicella			
Pneumococcal Conjugate[6]			PCV	PCV	PCV	PCV			PCV		PPV	
Influenza[7]					Influenza (Yearly)				Influenza (Yearly)			
Vaccines below red line are for selected populations												
Hepatitis A[8]									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 polysaccaride (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

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	57,686	(1969)	161	-99.72
Cong. Rubella Synd.	20,000+	(1964-5)	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 and year
+ Estimated because no national reporting existed in the prevaccine era
** Provisional

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 ($MSP1_{19}$)
    - RBC schizont antigen (SERA)
    - Gametocyte antigens (Pfs25)
  - Multiple Antigen Peptides (MAPs)
  - Strong adjuvants

Malaria, still on the horizon ?

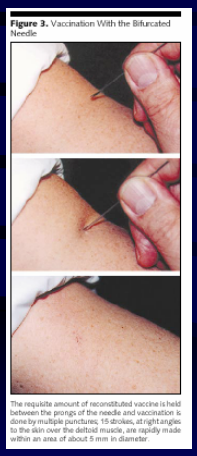
- Inadequate Long-term protection:
  - Failure to induce adequate memory T-cell responses?
  - Will prime-boost strategies work better?
- Additional references:
  - Targett, Trends in Parasitology, 2005
  - Okie, NEJM, 2005
  - http://www.who.int/vaccine_research/documents/en/malaria_table.pdf

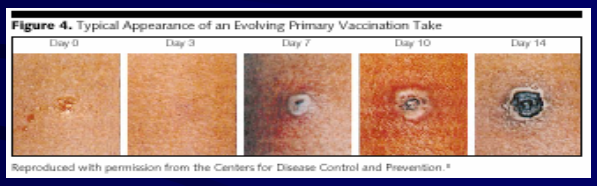
Additional Background Slides

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)

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

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

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

Can substitute
MDCK cells →

- **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

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
    - Intussuception:
      - 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 co-polymers)
  - Saponin derivatives
  - Polymers (polyphosphazene)
  - Bacterial toxins (cholera & E.coli HL)
    - Orally cholera toxin → T_h2 response → IgG1, IgE, mucosal IgA
  - Cytokines:
    - IL-6 → mucosal IgA & IgG
    - IL-4 → type 2 T-cell response (T_h2/ T_c2) → potent Ig production
    - IL-12 → type 1 T-cell response (T_h1/ T_c1) → 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_{197}: nontoxic mutant diphtheria toxin
- DTaP: Diphtheria, Tetanus, Pertussis (acellular)
- DTP: Diphtheria, Tetanus, Pertussis (whole cell)
- HbOC: a HIB vaccine that uses CRM_{197} as a carrier protein conjugated to PRP
- Hep A, Hep B: hepatitis A or B vaccine
- HIB: Hæmophilus *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 meningitis
- 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 polysaccaride 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