Vaccines

Robert Woodland
6-2465
Vaccines

• Lecture topics:
  • The benefits and risks of vaccination
  • The immunological basis for protection
  • Classes of vaccine antigens and adjuvants
  • Various tactics of vaccine development
  • Bad vaccine effects
  • Systems Biology and vaccine development
A Mini History Lesson

• The knowledge that previous infection can be protective
• Eastern and African cultures used variolation in the 16-17th century
• Ideas imported to the West in the 18th century
• Use of *attenuated pathogen* - Jesty 1770
• Application of the scientific method Jenner-1796
• Theory of induced protective immunity
• Lack of ethical concerns
Jenner and vaccine development

Jenner's scientific interests were varied, but the importance of his work in vaccination has overshadowed his other results. Early in his career he had begun to observe the phenomena of cowpox, a disease common in the rural parts of the western counties of England, and he was familiar with the belief, current among the peasantry, that a person who had suffered from the cowpox could not take smallpox.
Jenner and vaccine development

Jenner's scientific interests were varied, but the importance of his work in vaccination has overshadowed his other results. Early in his career he had begun to observe the phenomena of cowpox, a disease common in the rural parts of the western counties of England, and he was familiar with the belief, current among the peasantry, that a person who had suffered from the cowpox could not take smallpox. Finally, in 1796, he made his first experiment in vaccination, inoculating a boy of eight with cowpox, and, after his recovery, with smallpox; with the result that the boy did not take the latter disease.
Vaccination is the most effective method for disease prevention.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Maximum</th>
<th>1997</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diphtheria</td>
<td>206,939</td>
<td>4</td>
<td>99.99</td>
</tr>
<tr>
<td>Measles</td>
<td>894,134</td>
<td>138</td>
<td>99.98</td>
</tr>
<tr>
<td>Mumps</td>
<td>152,209</td>
<td>683</td>
<td>99.55</td>
</tr>
<tr>
<td>Pertussis (whooping cough)</td>
<td>265,269</td>
<td>6,564</td>
<td>97.52</td>
</tr>
<tr>
<td>Polio (paralytic)</td>
<td>21,269</td>
<td>0</td>
<td>100.00</td>
</tr>
<tr>
<td>Rubella</td>
<td>57,686</td>
<td>181</td>
<td>99.69</td>
</tr>
<tr>
<td>Congenital rubella syndrome</td>
<td>20,000*</td>
<td>5</td>
<td>99.98</td>
</tr>
<tr>
<td>Tetanus</td>
<td>1,560‡</td>
<td>50</td>
<td>96.79</td>
</tr>
<tr>
<td>Influenza (&lt;5 years)</td>
<td>20,000*</td>
<td>165</td>
<td>99.18</td>
</tr>
</tbody>
</table>

Data taken from REF. 5. * estimated; ‡ mortality.
Vaccination has eliminated naturally occurring smallpox and polio is almost eliminated.
Concerns about the safety of vaccines appeared early and often.
Vaccination can cause side effects

- **Inflammation and anaphylactic reactions** - usually from contaminants in the vaccine preparation—an example being egg proteins in flu vaccine or mercury containing preservatives.

- **Infection** - from improperly inactivated vaccine preparations or the use of a vaccine containing live-attenuated viruses in immunodeficient patients.

- **Neurological and autoimmune reactions** - perhaps by rare antigen cross reactions or perturbation of immunoregulatory circuits.

- **Lawyers** - fear of lawsuits is so prevalent that there are only three companies currently making vaccines. (Down from nine in the 80's.

- **Oprah** - has now decided that MMR vaccination is the cause of the increase in cases of autism.
Autism

- Recent studies have shown an increase in the frequency of autism.
- Thimerosal is a mercury containing compound used previously as a preservative in DTP, MMR and other vaccines.
- It has been hypothesized that autism is induced by heavy metal poisoning provoked by vaccination (thimersol).
- It has also been suggested that multiple infectious agents in vaccines induce a unique pathology.
- Most recently, it has been suggested that the age of the father is the greatest risk indicator.
The beginning of the autism scare

Ileal-lymphoid-nodular hyperplasia, non-specific colitis, and pervasive developmental disorder in children

A J Wakefield, S H Murch, A Anthony, J Linnell, D M Casson, M Malik, M Berelowitz, A P Dhillon, M A Thomson, P Harvey, A Valentine, S E Davies, J A Walker-Smith

We have identified a chronic enterocolitis in children that may be related to neuropsychiatric dysfunction. In most cases, onset of symptoms was after measles, mumps, and rubella immunisation. Further investigations are needed to examine this syndrome and its possible relation to this vaccine.
Wakefield tells GMC he was motivated by concern for children

Owen Dyer LONDON

The doctor at the centre of a major public health scare over vaccinations gave evidence this week before a General Medical Council panel, where he and two colleagues stand accused of research misconduct.

Andrew Wakefield, whose research paper and comments in 1998 linking the combined measles, mumps, and rubella (MMR) vaccine to autism led to a sharp fall in uptake of the vaccine, told the hearing that he was motivated by concern for autistic children.

He read out a letter that he had sent in 1997 to John Walker-Smith, now also accused of misconduct by the GMC, in which he wrote: “If these diseases are found to be linked to the MMR vaccine, these children are the few unfortunate who have been sacrificed to protect the majority.”

In that letter he defended his involvement with solicitors acting on behalf of parents seeking compensation from vaccine manufacturers. Six years after the publication of a study linking measles virus to irritable bowel syndrome and autism (Lancet 1998;351:637-41), it emerged that 10 of 12 patients involved in the research had legal aid backing to sue vaccine manufacturers and that the Legal Services Commission had funded his research (BMJ 2004;329:1293).

Dr Wakefield also received £435 643 (€550 000; $860 000) from the commission in fees to investigate and write reports on the safety of the MMR vaccine—fees that were not disclosed in the Lancet paper. When the payments became known in 2004 the Lancet repudiated the paper, and its editor, Richard Horton, said that the journal was compromised by a “fatal conflict of interest.”

Dr Wakefield denied that his research was motivated by legal or financial reasons. “The reason these parents were talking to me was nothing to do with the litigation, and litigation was not my primary concern.”

He told the hearing that when he was approached by lawyers representing the families of autistic children he had consulted the BMA to ask what the “going rate” was. “They indicated that the fee was £150 to £200 an hour,” he said. “I opted for the former of the two figures.”

Dr Wakefield also disputed allegations that while at the Royal Free Hospital, London, he conducted invasive tests, including colonoscopies and lumbar punctures, not approved by the hospital’s research ethics committee. Those tests were conducted for clinical, not research, purposes, he argued, and were beyond the remit of ethics approval.

Professor Walker-Smith, who is also facing the GMC panel accused of serious professional misconduct, decided which investigations were appropriate on the basis of clinical need, said Dr Wakefield.

A third consultant at Royal Free Hospital, Simon Murch, is also accused of participating in unapproved and unnecessary invasive procedures as part of the research. Professor Murch wrote to the Lancet in 2003 repudiating Dr Wakefield’s claims about MMR vaccine, calling him “completely wrong.”

His testimony continues.

See EDITORIAL p729
The “Vaccination Paradox” and public health

• As the pool of vaccinated individuals increases so does *herd immunity* (population protection due to a reduction in the reservoir of disease susceptible hosts) reducing disease even among the unvaccinated.

• As disease declines because of the efficiency of vaccination, the incidence of deleterious side effects becomes higher and the necessity for vaccination is questioned.
Cessation of vaccination can increase disease incidence

**Reduced MMR Equals More Measles**

Reduced uptake of the MMR vaccine, fueled no doubt by anti-vaccine propaganda, has resulted in a recent **significant increase in Measles in the UK** as shown by the graph on the right. And despite what the anti-vaccine twits will tell you, Measles can be a very serious disease. According to the **CDC**:

As many as one out of 20 children with measles gets pneumonia, and about one child in every 1,000 who get measles will develop encephalitis. (This is an inflammation of the brain that can lead to convulsions, and can leave your child deaf or mentally retarded.) For every 1,000 children who get measles, one or two will die from it.
How do vaccines work?
Humoral responses are specifically enhanced upon reexposure to the same (priming) antigen. This secondary response shows memory to the initial antigen.
B cell activation pathways and consequences

TI

Extra-follicular

TD

Extra-follicular

Germinal Center Reaction

TI

B D Ag

TD

Ag D B T

TD

Ag D B T

SHM
CSR restricted
Memory B Generation?
TFH
TFR
LLPC
Vaccination is an attempt to generate immunological memory without disease pathology

- Memory is induced through immune recognition of vaccine antigens.
- Immune memory allows an accelerated humoral and cellular responses if the vaccine antigen is re-encountered.
- Immune recognition leads to:
  - Neutralization of the infectious agent before it can enter cells
  - Destruction of infected cells before they multiply
  - Suppression of the spread of the infectious agent to other cells
The vaccine response and germinal centers

- The germinal center reaction provides an environment that promotes: the generation of short lived antibody forming cells; the induction of somatic hypermutation, affinity maturation by antigen selection and concurrent deletion of autoreactive cells; the production of immunological memory carried by long-lived antibody forming cells (humoral memory) and by long-lived recirculating small non-antibody secreting lymphocytes (classical precursor B memory cells).
- The chief accessory cells are follicular dendritic cells and T follicular helper cells
Cellular events during a T cell dependent response

Figure 1

Phase I
- Site of infection
- B-cell zone
- Primary follicle
- Ag-primed DC
- Ag-primed B cell
- Naive B cell
- Synapse I

Phase II
- Secondary follicle formation
- Ag-primed Th cell
- Ag-primed B cell
- Synapse II
- Plasma cell development

Phase III
- Memory
- GC
- Death
- Centrosome selection
- Light zone
- Retention
- Diversification of B
- Germinal center reaction
- Synapse III

Location
- T-cell zone
- B-cell zone
- Site of infection

Time
- Day 1
- Day 7

Bone marrow
Recirculate

Current Opinion in Immunology
Figure 1. Hypothetical model for the generation of short- and long-lived memory B cells and antibody-secreting cells in primary immune reactions. Short- and long-lived memory B cells
Mechanism of vaccine mediated protection:

- Produce B and T cell memory without disease
- B cell memory in the form of quiescent antigen specific recirculating cells.
- B cell memory in the form of long-lived antibody secreting cells residing in the bone marrow
- T (CD4) cells in the form of recirculating and bone marrow sequestered quiescent cells
Durable protection
Duration of Humoral Immunity to Common Viral and Vaccine Antigens

Ian J. Amanna, Ph.D., Nichole E. Carlson, Ph.D., and Mark K. Slifka, Ph.D.

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Durability of vaccine induced antibody responses

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Protective Titer</th>
<th>Subjects Protected†</th>
<th>Total Population</th>
<th>Antibody Half-Life‡</th>
<th>P Value</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>IU/ml</td>
<td>no. (%)</td>
<td></td>
<td></td>
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<tr>
<td>Tetanus</td>
<td>0.01</td>
<td>42 (93)</td>
<td>11 (10–14)</td>
<td>12 (10–16)</td>
<td>10 (8–14)</td>
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<tr>
<td>Diphtheria</td>
<td>0.01</td>
<td>40 (89)</td>
<td>19 (14–33)</td>
<td>26 (17–51)</td>
<td>14 (8–42)</td>
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<tr>
<td>VZV</td>
<td>NA</td>
<td>NA</td>
<td>50 (30–153)</td>
<td>63 (28–∞)</td>
<td>41 (23–212)</td>
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<tr>
<td>Vaccinia</td>
<td>3.8</td>
<td>28 (62)</td>
<td>92 (46–∞)</td>
<td>99 (48–∞)</td>
<td>85 (31–∞)</td>
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<tr>
<td>Rubella</td>
<td>10.0</td>
<td>39 (87)</td>
<td>114 (48–∞)</td>
<td>85 (43–∞)</td>
<td>190 (35–∞)</td>
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<tr>
<td>EBV</td>
<td>NA</td>
<td>NA</td>
<td>11,552 (63–∞)</td>
<td>No decay (84–∞)</td>
<td>3648 (35–∞)</td>
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<tr>
<td>Mumps</td>
<td>NA</td>
<td>NA</td>
<td>542 (90–∞)</td>
<td>124 (53–∞)</td>
<td>No decay (89–∞)</td>
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<tr>
<td>Measles</td>
<td>0.2</td>
<td>41 (91)</td>
<td>3014 (104–∞)</td>
<td>369 (67–∞)</td>
<td>No decay (74–∞)</td>
</tr>
</tbody>
</table>
Long term antibody production in four subjects
Serum antibody and memory B cells are sometimes discordant and it has been shown experimentally that Ab responses persist when memory B cells are depleted.
Types of vaccines
Types of Vaccine

- **Killed pathogen** - heat or formalin (Salk polio vaccine)
- **Live-attenuated** - selection of less or non pathogenic variants (Sabin Polio vaccine)
- **Subunit vaccine** - purified or genetically engineered structural component of a pathogen (Hepatitis B vaccine, Hep Bs Ag)
- **Secreted or extracted bacterial products** toxoids- (are toxins inactivated by chemical treatment or induced mutation to be immunogenic but not pathogenic); cell wall polysaccharides
- **Conjugate vaccines** - combination of multiple components to increase immunogenicity or memory induction
- **DNA vaccine** - inject the gene that makes the protein
- **Infectious agents** to accomplish gene delivery (recombinant vaccinia, adenovirus or bacteria)
- **Virus-like particles** genome free particles assembled from recombinant proteins expressing vaccine antigens.
What are the key attributes of an ideal vaccine?

<table>
<thead>
<tr>
<th>Features of effective vaccines</th>
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<tbody>
<tr>
<td>Safe</td>
<td>Vaccine must not itself cause illness or death</td>
</tr>
<tr>
<td>Protective</td>
<td>Vaccine must protect against illness resulting from exposure to live pathogen</td>
</tr>
<tr>
<td>Gives sustained protection</td>
<td>Protection against illness must last for several years</td>
</tr>
<tr>
<td>Induces neutralizing antibody</td>
<td>Some pathogens (such as poliovirus) infect cells that cannot be replaced (e.g., neurons). Neutralizing antibody is essential to prevent infection of such cells</td>
</tr>
<tr>
<td>Induces protective T cells</td>
<td>Some pathogens, particularly intracellular, are more effectively dealt with by cell-mediated responses</td>
</tr>
<tr>
<td>Practical considerations</td>
<td>Low cost-per-dose, Biological stability, Ease of administration, Few side-effects</td>
</tr>
</tbody>
</table>

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Vaccines

Infectious agent
Toxic product

- Inactivate
- Attenuate

Subunit protein or polysaccharide
- Polio
- Hepatitis A
- HPV
- RSV
- Diptheria toxin
- Tetanus toxin
- Pox-HIV
- HIV
- Hepatitis B
- Pneumovax 23
- VLP

Conjugates Polysaccharide Protein
- Hemophilus polysaccharide-protein conjugate
- Pneumococcus polysaccharide-protein conjugate

DNA encoded proteins
- HIV
- SARS
Attenuation of pathogenic viruses by growth in non-human cells (Rabies and Pasteur)
Preparation of conjugates to make T-independent antigens

T dependent *

Conjugate vaccines facilitate T and B cell collaboration
Jenner’s success was made possible by antigens shared by cowpox and smallpox
**Adjuvants**

<table>
<thead>
<tr>
<th>Adjuvant name</th>
<th>Composition</th>
<th>Mechanism of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incomplete Freund's adjuvant</td>
<td>Oil-in-water emulsion</td>
<td>Delayed release of antigen; enhanced uptake by macrophages</td>
</tr>
<tr>
<td>Complete Freund's adjuvant</td>
<td>Oil-in-water emulsion with dead mycobacteria</td>
<td>Delayed release of antigen; enhanced uptake by macrophages; induction of co-stimulators in macrophages</td>
</tr>
<tr>
<td>Freund's adjuvant with MDP</td>
<td>Oil-in-water emulsion with muramyl dipeptide (MDP), a constituent of mycobacteria</td>
<td>Similar to complete Freund's adjuvant</td>
</tr>
<tr>
<td>Alum (aluminum hydroxide)</td>
<td>Aluminum hydroxide gel</td>
<td>Delayed release of antigen; enhanced macrophage uptake</td>
</tr>
<tr>
<td>Alum plus <em>Bordetella pertussis</em></td>
<td>Aluminum hydroxide gel with killed <em>B. pertussis</em></td>
<td>Delayed release of antigen; enhanced uptake by macrophages; induction of co-stimulators</td>
</tr>
<tr>
<td>Immune stimulatory complexes (ISCOMs)</td>
<td>Matrix of Quil A containing viral proteins</td>
<td>Delivers antigen to cytospot; allows induction of cytotoxic T cells</td>
</tr>
</tbody>
</table>

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Stimulation through pattern recognition receptors (PRRs) primes the innate immune system and improves adaptive responses.
<table>
<thead>
<tr>
<th>Pattern-recognition receptors</th>
<th>Ligands</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR1(^a)</td>
<td>Bacterial lipoproteins from <em>Mycobacteria</em>, <em>Neisseria</em></td>
</tr>
<tr>
<td>TLR2(^a)</td>
<td>Zymosan yeast particles, peptidoglycan, lipoproteins, glycolipids, lipopolysaccharide</td>
</tr>
<tr>
<td>TLR3</td>
<td>Viral double-stranded RNA, poly:IC</td>
</tr>
<tr>
<td>TLR4</td>
<td>Bacterial lipopolysaccharides, plant product taxol</td>
</tr>
<tr>
<td>TLR5</td>
<td>Bacterial flagellins</td>
</tr>
<tr>
<td>TLR6(^a)</td>
<td>Yeast zymosan particles, lipotechoic acid, lipopeptides from mycoplasma</td>
</tr>
<tr>
<td>TLR7</td>
<td>Single-stranded RNA, R-837 and R848(^b), other synthetic compounds such as loxoribine and boprimine</td>
</tr>
<tr>
<td>TLR8(^c)</td>
<td>Single-stranded RNA, R848</td>
</tr>
<tr>
<td>TLR9(^d)</td>
<td>CpG oligonucleotides</td>
</tr>
<tr>
<td>TLR10</td>
<td>Unknown</td>
</tr>
<tr>
<td>TLR11</td>
<td>Bacterial components from uropathogenic bacteria</td>
</tr>
<tr>
<td>NOD1, NOD2(^e)</td>
<td>Peptidoglycans</td>
</tr>
<tr>
<td>Scavenger receptors(^f)</td>
<td>Acetylated/maleylated proteins; modified low-density lipoproteins and other polyanionic ligands</td>
</tr>
<tr>
<td>Macrophage mannose receptors and other c-type lectin receptors</td>
<td>Sulfated sugars, mannose-, fucose- and galactose-modified polysaccharides and proteins</td>
</tr>
<tr>
<td>Type 3 complement receptors and dectin type receptors</td>
<td>Zymosan particles, β-glucan</td>
</tr>
</tbody>
</table>

\(^a\)TLRs can form heterodimers, which further changes their specificity. For example, TLR2 and TLR6 form heterodimers that recognize a mycoplasmal lipoprotein\(^44\). TLR1 and TLR2 have similarly been shown to cooperatively recognize a mycobacterial lipoprotein\(^45\). \(^b\)R-837 (imiquimod) and R-848 (resiquimod) are the first small-molecule synthetic TLR ligands to be.
**Fig. 1.** The activation of T-helper cells (Th1 and Th2) requires specific signals from an antigen presenting cell (APC). There are several targets for adjuvants to interact with this process and to modulate the adaptive immune response. Adjuvants can impact at different levels such as: (1) Recognition of a pathogen associated molecular pattern (PAMP) by a toll-like receptor (TLR). (2) Presentation of antigens by the major histocompatibility complex (MHC) to the T-cell receptor (TCR). (3) Recognition of co-stimulatory signals like CD28 or CD40L by specific T-cell CD80/86 and CD40 ligands. (4) Intracellular signalling processes in the APC.
$T_{FH}$ cells are a unique helper T cell population with high antigen binding potential.
Adjuvants affect the quantity and quality of $T_{FH}$ cells
Strategies for making better vaccines and more effective vaccine delivery
Antigen and adjuvant delivery

Unbroken Skin
Adjuvants: CT; LT; CpG; Imiquimod; LPS or lipid A

Particles: Attenuated virus; ISCOMs; nanoparticles; transfersomes; microemulsions

After abrasion or direct penetration
Proteins; DNA; Vectors; Cells; Powder with antigen; Liposomes with antigen; Liquids with antigen

Hydrated patch

Micro needles
Emery paper

Stratum corneum
Langerhan’s cells
Epidermis

Stratum lucidum
Stratum granulosum
Stratum spinosum
Stratum basale

Vigorous tape stripping or other physical treatment

Current Opinion in Immunology
Case 1 pneumococcal vaccines

- Pneumococcal disease is treatable, penicillin and trimethoprim sulfate are the drugs of choice.
  But - widespread antimicrobial resistance

- 90 capsular serotypes based on antiphagocytic capsule.
  Each Serotype ---- > 46 serogroups based on the structural and chemical composition of polysaccharides

- Pneumococci acquire new phenotypic traits through natural transformation, frequent recombination between individual strains allows gene mosaicism

- Estimated that pneumococci possess over 100 surface proteins, many of which play a role in pathogenicity and virulence
Discovery of a novel class of highly conserved vaccine antigens using genomic scale antigenic fingerprinting of pneumococcus with human antibodies


Journal of Experimental Medicine
Volume 205(1):117-131
January 2, 2008
Characterization and selection of human sera for antigen identification

Peptide ELISA with human sera

Giefing et al. Journal of Experimental Medicine
2008:205:117-131
Selection of antigenic pneumococcal proteins

A

PcsB (392 aa)

GLU_RICH

PspC (693 aa)

B

Proteome

ANTIGENome

Giefing et al. Journal of Experimental Medicine
2008:205:117-131

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PcsB and StkP are highly conserved antigens
Protection by PcsB and StkP in models of pneumococcal sepsis and pneumonia

Giefing et al. Journal of Experimental Medicine
2008:205:117-131

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Characterization of ΔpcsB and ΔstkP gene deletion mutant strains

A

<table>
<thead>
<tr>
<th>Drug</th>
<th>ΔstkP (%)</th>
<th>ΔpcsB (%)</th>
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<tbody>
<tr>
<td>Vancomycin</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>80</td>
<td>60</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>60</td>
<td>80</td>
</tr>
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</table>

B

WT: [Image of WT cells]
ΔstkP: [Image of ΔstkP cells]
ΔpcsB: [Image of ΔpcsB cells]

Giefing et al. Journal of Experimental Medicine
2008:205:117-131
StkP and PcsB antigens are immunogenic in young children. 88 sera obtained from children not suffering from any infectious disease at the time of sampling were analyzed for antipneumococcal IgG levels by ELISA using recombinant proteins as coating antigens.


© 2008 Giefing et al.
Antigen-specific immune sera show surface staining and OPK activity

Case 2 HIV vaccine
Thailand HIV Trial

- Canarypox-HIVgp120 prime (AID/VAX)
- Boost recombinant gp120 subunit vaccine
New efforts to produce a vaccine against HIV

Defining target epitopes that facilitate the production of broadly cross reactive neutralizing antibody

- Identify patients with neutralizing antibody
- Isolate memory B cells by flow cytometry
- Distribute cells ~1/well and activate by polyclonal stimulation
- Test for neutralization on a small virus panel
- Use PCR to isolate Ig gene segments for H and L chains
- Express Ig segment clones in a producer cell line
- Identify target epitopes
- Use these insights to produce a vaccine.
The isolation of broadly neutralizing antibodies to HIV using memory cell selection and Ig cloning strategy

<table>
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<tr>
<th>Clade</th>
<th>No. of viruses</th>
<th>Median IC(<em>{50}) (µg/ml) against viruses neutralized with an IC(</em>{50}) &lt;50 µg/ml</th>
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<tr>
<td></td>
<td></td>
<td>b12</td>
</tr>
<tr>
<td>A</td>
<td>27</td>
<td>6.98</td>
</tr>
<tr>
<td>B</td>
<td>31</td>
<td>0.80</td>
</tr>
<tr>
<td>C</td>
<td>27</td>
<td>6.46</td>
</tr>
<tr>
<td>D</td>
<td>25</td>
<td>1.47</td>
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<td>CRF01_AE</td>
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<td>21.53</td>
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<tr>
<td>CRF_AG</td>
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<td>10.40</td>
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<tr>
<td>G</td>
<td>15</td>
<td>3.07</td>
</tr>
<tr>
<td>F</td>
<td>15</td>
<td>&gt;50</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>162</strong></td>
<td>2.82</td>
</tr>
<tr>
<td>Clade</td>
<td>No. of viruses</td>
<td>b12</td>
</tr>
<tr>
<td>-------------</td>
<td>----------------</td>
<td>-----</td>
</tr>
<tr>
<td>A</td>
<td>27</td>
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</tr>
<tr>
<td>B</td>
<td>31</td>
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<tr>
<td>C</td>
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<td>D</td>
<td>25</td>
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With an IC$_{50}$ < 50 μg/ml

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With an IC$_{50}$ < 1.0 μg/ml
Detection of effective antibodies may require assays using native ENV structures
Figure 1  Structure and Antibody Recognition of the HIV Envelope Spike  The molecule is a heterotrimer of composition (gp120) 3 (gp41) 3 . Gp41 is a transmembrane protein, and gp120 is the receptor molecule for CD4 and CCR5 (or CXCR4). The model ...

Dennis R. Burton, Rafi Ahmed, Dan H. Barouch, Salvatore T. Butera, Shane Crotty, Adam Godzik, Daniel E. Kau...

**A Blueprint for HIV Vaccine Discovery**

Cell Host & Microbe Volume 12, Issue 4 2012 396 - 407

http://dx.doi.org/10.1016/j.chom.2012.09.008
Co-merging the concepts of breath and potency

A Blueprint for HIV Vaccine Discovery

Cell Host & Microbe Volume 12, Issue 4 2012 396 - 407

http://dx.doi.org/10.1016/j.chom.2012.09.008
Case 3. Modified bacteria as vaccines
Killed but metabolically active microbes: a new vaccine paradigm for eliciting effector T-cell responses and protective immunity

D G Brockstedt¹,⁵, K S Bahjat¹,⁵, M A Giedlin¹,⁵, W Liu¹, M Leong¹, W Luckett¹, Y Gao¹, P Schnupf², D Kapadia¹, G Castro¹, J Y H Lim¹, A Sampson-Johannes¹, A A Herskovits², A Stassinopoulos¹, H G Archie Bouwer², J E Hearst¹, D A Portnoy²,⁴, D N Cook¹ & T W Dubensky, Jr.¹

We developed a new class of vaccines, based on killed but metabolically active (KBMA) bacteria, that simultaneously takes advantage of the potency of live vaccines and the safety of killed vaccines. We removed genes required for nucleotide excision repair (uvrAB), rendering microbial-based vaccines exquisitely sensitive to photochemical inactivation with psoralen and long-wavelength ultraviolet light. Colony formation of the nucleotide excision repair mutants was blocked by infrequent, randomly distributed psoralen crosslinks, but the bacterial population was able to express its genes, synthesize and secrete proteins. Using the intracellular pathogen Listeria monocytogenes as a model platform, recombinant psoralen-inactivated Lm ΔuvrAB vaccines induced potent CD4⁺ and CD8⁺ T-cell responses and protected mice against virus challenge in an infectious disease model and provided therapeutic benefit in a mouse cancer model. Microbial KBMA vaccines used either as a recombinant vaccine platform or as a modified form of the pathogen itself may have broad use for the treatment of infectious disease and cancer.
Deletion of key genes makes a bacterium replication incompetent but viable and metabolically active.
Induction of T cell responses to a recombinant antigen OVA
Protection against recombinant vaccinia

(a) Vaccine strain and vaccine treatment

<table>
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<tr>
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<th>Live 1x</th>
<th>Heat-killed 1x</th>
<th>S-59/UVA 1x</th>
<th>S-59/UVA 3x</th>
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(b) Log FFU/ml vs. Vaccination

(c) Percent OVA-specific CD8 T cells

P < 0.05
Protection against Listeria
Protection against tumor cell challenge following immunization with Listeria expressing a recombinant modified self protein

<table>
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<th>Vaccine treatment</th>
<th>Listeria vaccine strain</th>
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<td>Live</td>
<td>Lm ΔactA (42 ± 17)</td>
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<td></td>
<td>Lm ΔactA-AH1-A5 (48 ± 9)</td>
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<td>S-59/UVA</td>
<td>Lm ΔactAΔuvrAB-AH1-A5 (4 ± 3)</td>
</tr>
<tr>
<td>Heat-killed</td>
<td>Lm ΔactAΔuvrAB-AH1-A5 (79 ± 24)</td>
</tr>
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</table>

**b**

![Graph showing percent survival vs. days after tumor implant](image)

- **Vaccine**: HBSS, Lm ΔactAΔuvrAB, Lm ΔactA-AH1-A5, Lm ΔactA-AH1-A5
- **Treatment**: Live, Heat-killed, S59/UVA

\[ P = 0.0041 \]
Case 4 Inducing immunity with DNA vaccines
Antigen priming from a DNA vaccine

- DNA
- protein
- MHC I
- MHC II
- Other APC
- B cells
- CD8^+
- CD4^+
Control of a Mucosal Challenge and Prevention of AIDS by a Multiprotein DNA/MVA Vaccine

Rama Rao Amara,1,3 Francois Villinger,2 John D. Altman,3 Shari L. Lydy,1,3 Shawn P. O’Neil,1,3 Silvija I. Staprans,6 David C. Montefiori,4 Yan Xu,1 James G. Herndon,1 Linda S. Wyatt,5 Maria Angelito Candido,1* Natalia L. Kozyr,6 Patricia L. Earl,5 James M. Smith,1,3 Hak-Ling Ma,1,7† Bennett D. Grimm,2 Michael L. Hulsey,3 Joseph Miller,3 Harold M. McClure,1 Janet M. McNicholl,7 Bernard Moss,5 Harriet L. Robinson1,3‡

Heterologous prime/boost regimens have the potential for raising high levels of immune responses. Here we report that DNA priming followed by a recombinant modified vaccinia Ankara (rMVA) booster controlled a highly pathogenic immunodeficiency virus challenge in a rhesus macaque model. Both the DNA and rMVA components of the vaccine expressed multiple immunodeficiency virus proteins. Two DNA inoculations at 0 and 8 weeks and a single rMVA booster at 24 weeks effectively controlled an intrarectal challenge administered 7 months after the booster. These findings provide hope that a relatively simple multiprotein DNA/MVA vaccine can help to control the acquired immune deficiency syndrome epidemic.
DNA gag+env → Vaccinia Gag+Env → Virus challenge
Outcomes in infected and immunized or control monkeys

Fig. 2. Temporal viral loads, CD4 counts, and survival after challenge of vaccinated and control animals. (A) Geometric mean viral loads and (B) geometric mean CD4 counts. (C) Survival curve for vaccinated and control animals. The dotted line represents all 24 vaccinated animals. (D) Viral loads and (E) CD4 counts for individual animals in the vaccine and control groups. The key to animal numbers is presented in (E). Assays for the first 12 weeks after challenge had a detection level of 1000 copies of RNA per milliliter of plasma. Animals with loads below 1000 were scored with a load of 500. For weeks 16 and 20, the detection level was 300 copies of RNA per milliliter. Animals with levels of virus below 300 were scored at 300.
Fig. 4. Lymph node histomorphology at 12 weeks after challenge. (A) Typical lymph node from a vaccinated macaque showing evidence of follicular hyperplasia characterized by the presence of numerous secondary follicles with expanded germinal centers and discrete dark and light zones. (B) Typical lymph node from an infected control animal showing follicular depletion and paracortical lymphocellular atrophy. (C) A representative lymph node from an age-matched, uninfected macaque displaying nonreactive germinal centers. (D) The percentage of the total lymph node area occupied by germinal centers was measured to give a nonspecific indicator of follicular hyperplasia. Data for uninfected controls are for four age-matched rhesus macaques.
T cell induced responses

Tetramer staining or intracellular IFN-γ

Fig. 3. Postchallenge T cell responses in vaccine and control groups. (A) Temporal tetramer+ cells (dashed blue line) and viral loads (solid pink line). (B) Intracellular cytokine assays for IFN-γ production in response to stimulation with the Gag-CM9 peptide at 2 weeks after challenge. This ex vivo assay allows evaluation of the functional status of the peak postchallenge tetramer+ cells displayed in Fig. 1A. (C) Proliferation assay at 12 weeks after challenge. Gag-Pol-Env (open bars) and Gag-Pol (hatched bars) produced by transient transfections were used for stimulation. Supernatants from mock-transfected cultures served as
Antibody responses following immunization and challenge

Fig. 5. Temporal antibody responses. Micrograms of total Gag (A) or Env (B) antibody were determined with ELISAs. The titers of neutralizing antibody for SHIV-89.6 (C) and SHIV-89.6P (D) were determined with MT-2 cell killing and neutral red staining (29). Titers are the reciprocal of the serum dilution giving 50% neutralization of the indicated viruses grown in human PBMC. Symbols for animals are the same as in Fig. 2.
<table>
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<th>Strategy</th>
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<td>Self-replicating viral replicons</td>
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<td>Codon optimization</td>
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<td><em>In vivo</em> electroporation</td>
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<td>Incorporation of genes for cytokines and co-stimulatory molecules</td>
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<tr>
<td>Incorporation of genes for chemokines</td>
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<td>Incorporation of additional CpG stimulatory motifs</td>
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<td>Targeting of the endocytic or ubiquitin-processing pathways</td>
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<td>Prime–boost regimens</td>
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<td>Use of mucosal delivery vectors (for example, <em>Salmonella</em>)</td>
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Bad vaccines
Bad vaccines:
Respiratory syncytial virus

- In the 1980s a formalin inactivated RSV was used in vaccine trials.
- Dozens of children immunized with this vaccine died of respiratory disease when infected with RSV.
- Vaccination had enhanced susceptibility to disease.
- What went wrong?
Lack of antibody affinity maturation due to poor Toll-like receptor stimulation leads to enhanced respiratory syncytial virus disease

Maria Florencia Delgado¹, Silvina Coviello¹, A Clara Monsalvo¹, Guillermina A Melendi¹,², Johanna Zea Hernandez¹,², Juan P Bataille¹, Leandro Diaz¹, Alfonzina Trento³, Herng-Yu Chang⁴, Wayne Mitzner⁴, Jeffrey Ravetch⁵, José A Melero³, Pablo M Irusta¹,⁶ & Fernando P Polack¹,²,⁷,⁸
Immunization strategy effects ERD

Vaccine ERD: Low affinity antibody is induced by formalin treated virus.

Figure 2 Nonreplicating vaccine elicits non-protective, low avidity antibody. (a) IgG antibody responses against the RSV F protein determined by
Protection against RSV and ERD is dependent on MyD88

Analysis of DC from draining LN after foot pad injections\textsuperscript{a}.
Germinal center responses in RSV or UVRSV treated mice

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<tr>
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![Images showing germinal center responses with PNA+ markers over time and across different treatments.](image-url)
Immunization with UViRSV plus TLR agonists protects against ERD
Passive protection against RSV and ERD using antibodies from donors immunized with UViRSV and TLR agonists
Impaired TLR signaling a cause of RSV vaccine enhanced disease?

F protein
Newcastle Disease Virus-Like Particles Containing Respiratory Syncytial Virus G Protein Induced Protection in BALB/c Mice, with No Evidence of Immunopathology


Virus like particle (VLP) synthesized using a chimeric HN/G protein
Immunization with VLPs produces long-lived protective antibodies
VLP immunization produces protection without ERD upon RSV challenge
VLP immunization produces protection without ERD upon RSV challenge
Vaccination against influenza
Vaccination against influenza

- Influenza presents unique problems. Generation of new viruses is rapid with birds and pigs providing an enormous reservoir of genetic material for virus recombination.
- Error prone replication constantly produces new virus “species”.
- Selection of virus variants for seasonal vaccine production is not precise resulting in the production of large vaccine stocks lacking the most prevalent virus.
- Varying numbers of vaccine recipients fail to respond to the relevant virus antigens.
- These considerations have prompted a search for vaccine preparations that can elicit broadly neutralizing (multi-seasonal) antibodies.
Structure of influenza hemagglutinin
A Strategy for producing influenza anti-stalk antibodies

B

Phil82

% initial weight

110

100

90

80

70

60

0

1

2

3

4

5

6

7

8

9

10

11

12

13

14

Days post challenge

% survival

100

90

80

70

60

0

1

2

3

4

5

6

7

8

9

10

11

12

13

14

Days post challenge

***, p=0.0008

C

Phil82

D

X-31

% initial weight

110

100

90

80

70

60

0

1

2

3

4

5

6

7

8

9

10

11

12

13

14

Days post challenge

% survival

100

90

80

70

60

0

1

2

3

4

5

6

7

8

9

10

11

12

13

14

Days post challenge

***, p=0.0001

E

X-31
Producing anti-stalk antibodies by boosting preexisting anti influenza responses
Too good to be true?
Negative effects of vaccination

**Vaccine-Induced Anti-HA2 Antibodies Promote Virus Fusion and Enhance Influenza Virus Respiratory Disease**
Surender Khurana *et al.*
*Sci Transl Med* 5, 200ra114 (2013);
DOI: 10.1126/scitranslmed.3006366

The authors vaccinated pigs with whole inactivated H1N2 influenza virus. These pigs had enhanced pneumonia and disease after infection with another strain — pH1N1. Looking more closely, the authors found that the immune sera from the H1N2-vaccinated pigs contained high titers of cross-reactive hemagglutinin antibodies. These antibodies actually enhanced pH1N1 infection in cell culture by promoting virus membrane fusion activity, and this enhanced fusion correlated with lung pathology. This mechanism of VAERD should be considered when devising strategies to devise a universal flu vaccine.
Antibody (vaccine) induced enhancement of infection

Fig. 1. Potential mechanisms for antibody-enhanced disease. (A) Enhanced fusion. Binding of antibody to the viral membrane receptor facilitates fusion of viral envelope with the cell membrane. (B) Virion attachment to Fc receptor on antigen-presenting cell. (C) Complement binding to infected epithelial cell.
Can we predict what vaccines are effective and what proportion of the population will be responsive?
Systems Vaccinology:

- Attempts to identify molecular signatures that can predict vaccine efficacy.
- Blood samples from vaccinated patients provide information on cytokine induction and antibody production as well as lymphocytes for use in determining T cell responses.
- The genetic analysis utilizes purified RNA from the PBMCs of newly vaccinated patients for determining transcription profiles using DNA arrays or RNA sequencing technologies.
- Limited ability to compare alternative vaccine formulations.
- Need large sample sizes due to human genetic variation.
- Results impacted by previous immune history.
The first modeled vaccine YF-17D

TLR, Toll-like receptor; WHO, World Health Organization.

The response to YF-17D

Overview of analysis

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<th><strong>Input</strong></th>
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</table>

H. Nakaya, S. Li and B. Pulendran

Analytical approach
Quantitative assessment of gene expression and subsequent pathway organization.
Establishing antibody response phenotype using gene profiling
Using systems biology to assess vaccine efficacy
Predicting the unknown from the known.
Induction of ICOS⁺CXCR3⁺CXCR5⁺ T_H Cells Correlates with Antibody Responses to Influenza Vaccination

Salah-Eddine Bentebibel,¹,²* Santiago Lopez,³* Gerlinde Obermoser,¹* Nathalie Schmitt,¹* Cynthia Mueller,¹ Carson Harrod,¹,² Emilio Flano,³ Asuncion Mejias,³ Randy A. Albrecht,⁴,⁵ Derek Blankenship,¹ Hui Xu,¹ Virginia Pascual,¹,² Jacques Banchereau,¹ Adolfo Garcia-Sastre,⁴,⁵,⁶ Anna Karolina Palucka,¹,² Octavio Ramilo,³† Hideki Ueno¹,²†
Immunization with flu vaccine induces $T_{F-H}$ cells
Relationship between antibody titer and $T_{FH}$ cell number
TFH cells are uniquely effective at providing help for memory B cells to produce anti-influenza antibody.

**Fig. 8.** ICOS⁺CXCR3⁺CXCR5⁺CD4⁺ T cells induce antigen-specific antibody response. (A) Proliferation of
The microbiome influences the induction of protective immunity to influenza infection.

Fig. 1. Antibiotic-treated mice fail to induce acquired immunity to influenza virus infection. C57BL/6 mice were given ampicillin (1 g/L), vancomycin (500 mg/L), neomycin sulfate (1 g/L), and metronidazole (1 g/L) in drinking water for 4 wk before PR8 virus infection (10 pfu per mouse). Two weeks later, serum and nasal wash were collected and Ag-specific antibody titers were measured (A), and T-cells were isolated from spleen and restimulated with flu virion or NP peptide for 72 h, and IFN-γ production from CD4 T cells (B) and CD8 T cells (C) was measured. (D) Lymphocytes were collected from the lung of infected animals at 14 d postinfection and stained with flu-specific tetramer. (E) The lung washes of flu-infected mice were harvested at 9 d postinfection, and viral titers were measured by plaque assay. *P < 0.05 and ***P < 0.001 vs. water-fed group. Data represent the mean ± SD. Similar results were obtained from three separate experiments.
The final question is can you predict protection against disease?
Getting a vaccine to the patient
That’s All