New Technologies and Vaccine Development

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Courtesy of T Sharrar, Smithsonian Institution

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Need for New Vaccines

Disease	Annual New Cases *(Million)*	Annual Deaths *(Million)*
Diarrheal Diseases	1,300	2.5-4
Acute Respiratory Diseases		3.7
Tuberculosis	7-8	2-3
HIV	5.8	3
Malaria	500	1.5-3

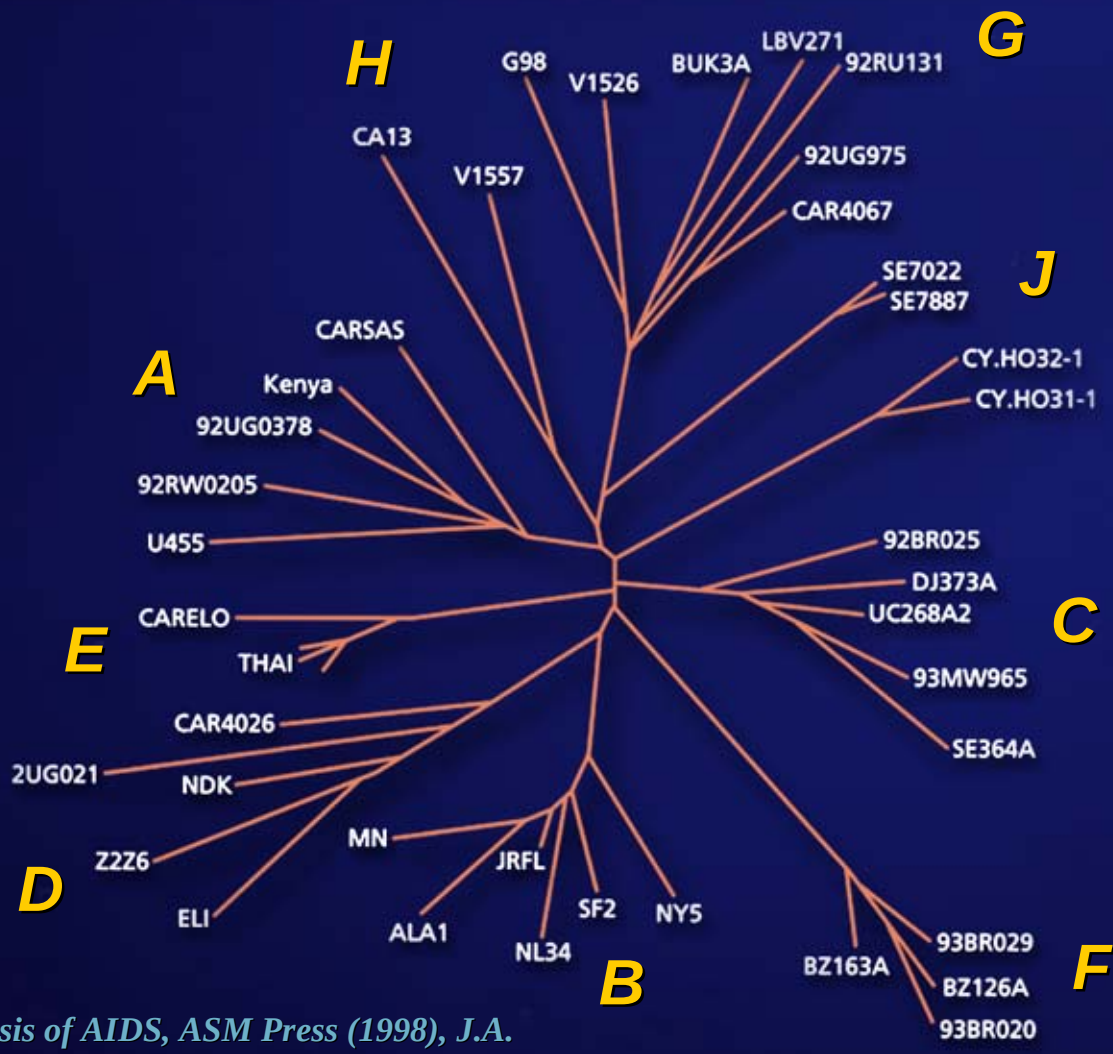
Issues for Live Attenuated Virus Vaccines

- Natural infection may not induce immunity or optimal immune responses
- Some viruses cause deleterious immune responses
- Potential reversion to virulence
  - Concern for HIV
- Decreased efficacy due to pre-existing antibodies
  - Influenza
- Decoy antigens on the virus

Comparison of Vaccine Technologies

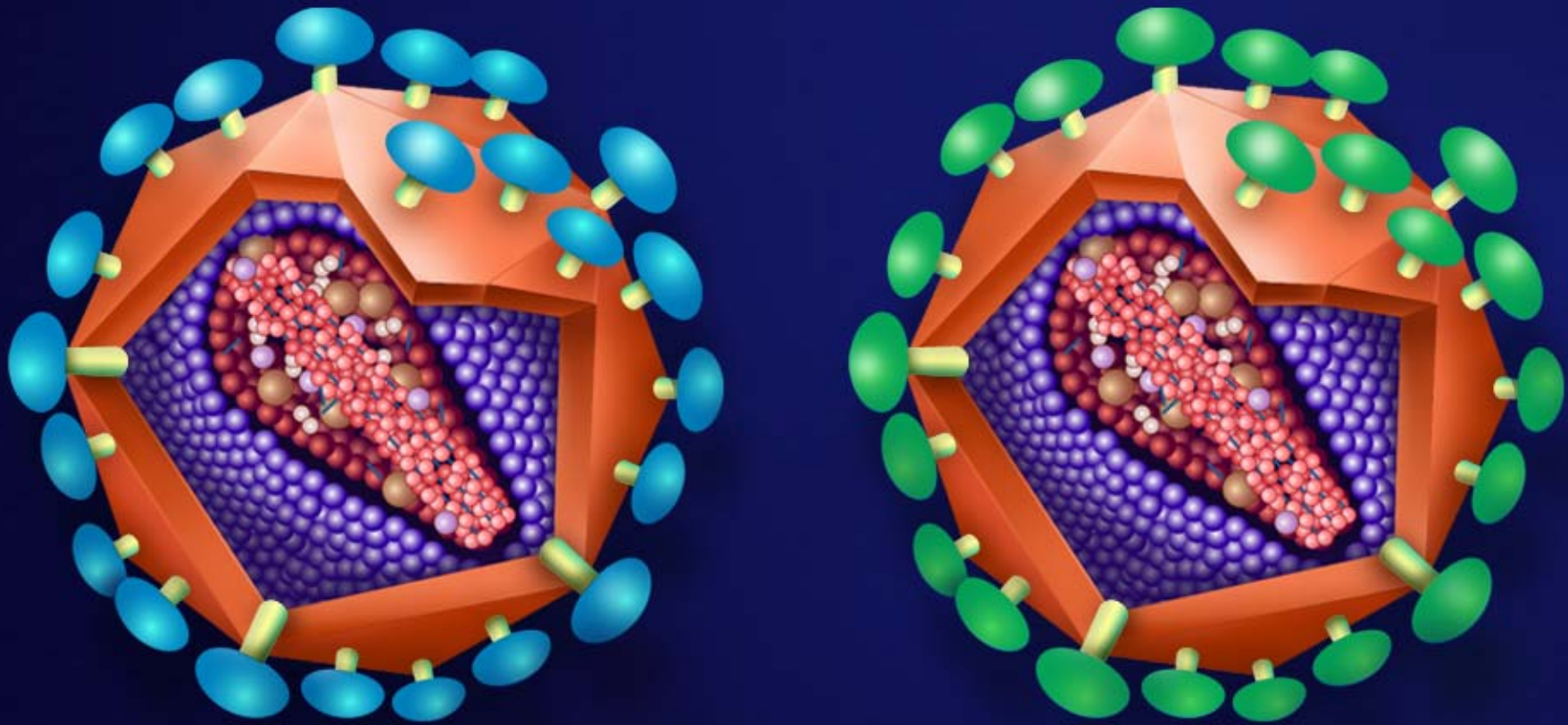
- Live attenuated viruses
  - Highly effective
  - Potential risk
  - Manufacturing challenge
- Recombinant proteins
  - Potent antibody response
  - Non-native forms
  - Not induce CTL
- Viral vectors
  - Risk
  - Resistance / pre-existing antibody
  - Inflammation
- DNA vaccines
  - Need for increased potency
  - Designer immune response e.g., Type of T_H
  - Specificity: avoid deleterious or diversional antigens
  - Stability
  - Safety
  - Generic manufacturing
  - Cost

HIV Clade (Strain) Diversity

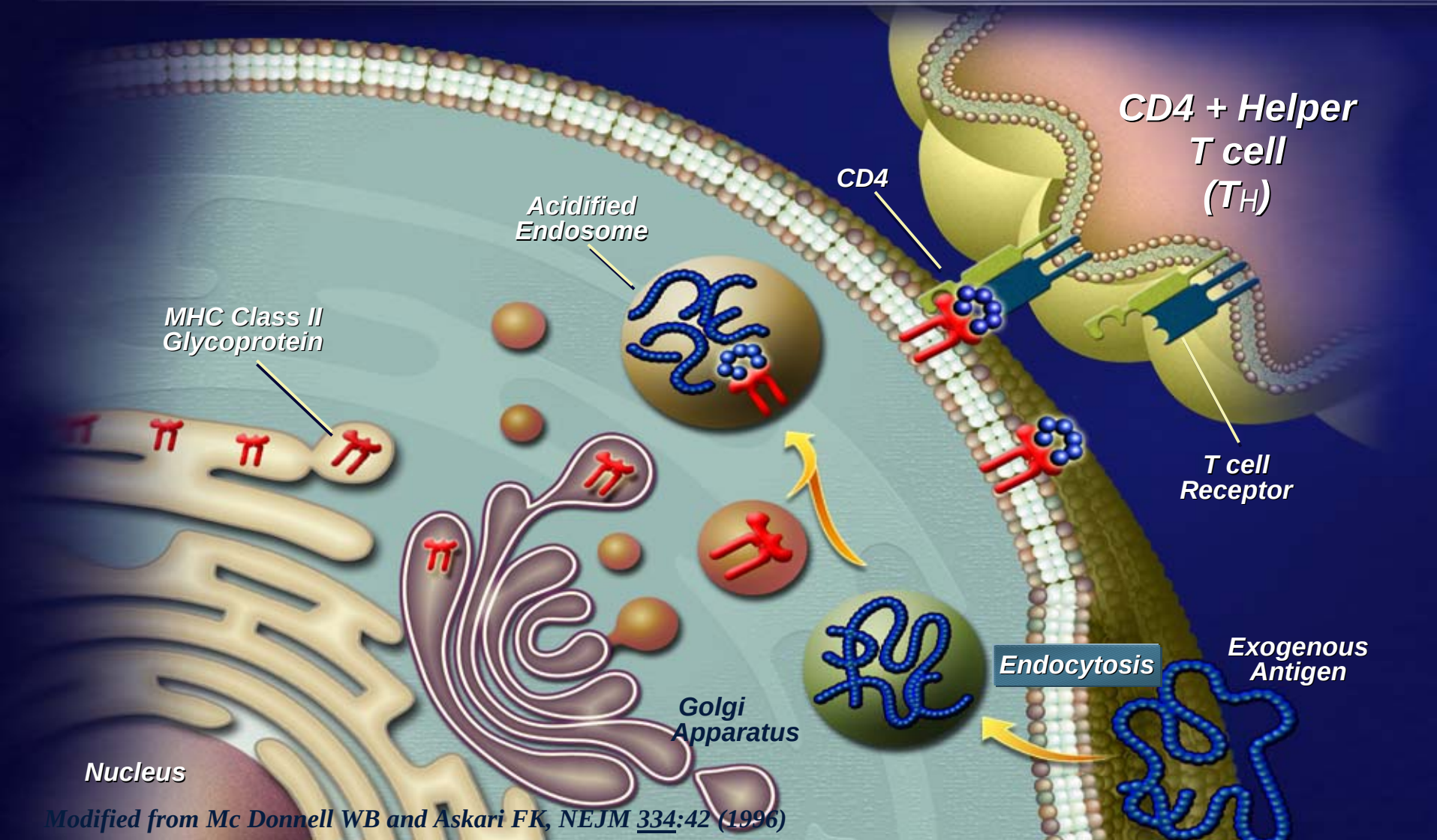


HIV and the Pathogenesis of AIDS, ASM Press (1998), J.A. Levy

Heterogeneity of HIV Strains

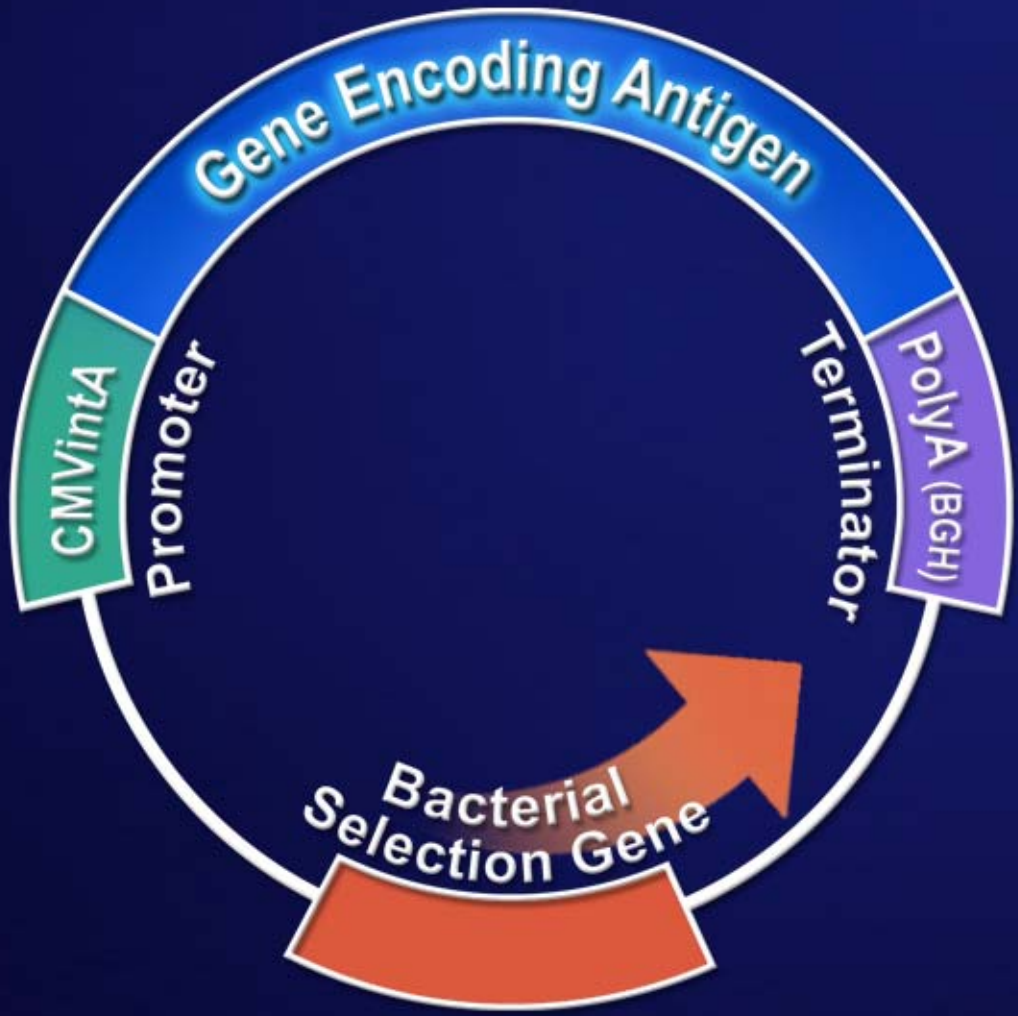


Exogenous Protein Results in Generation of T Cell Help But Not CTL

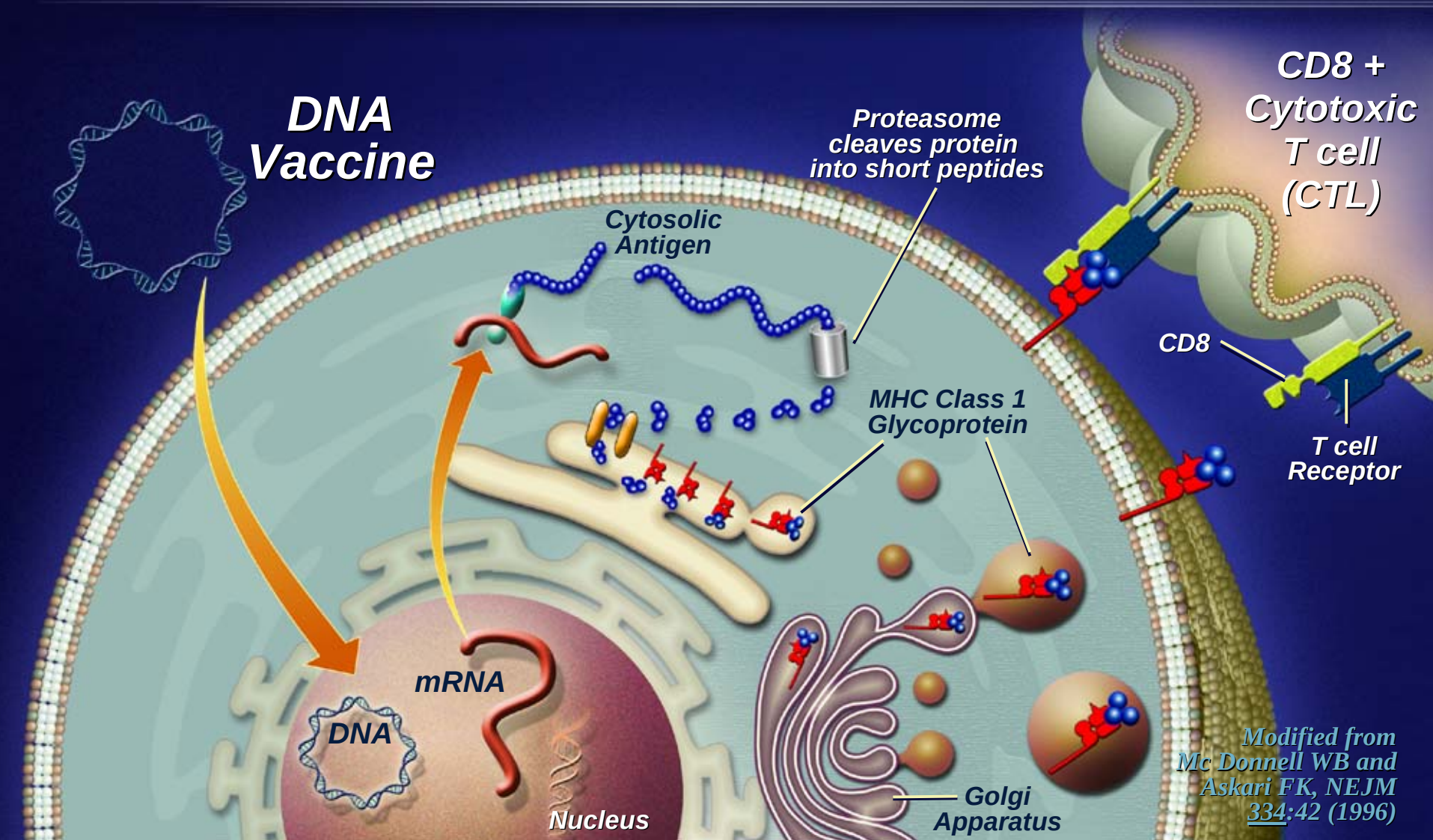


Modified from Mc Donnell WB and Askari FK, NEJM 334:42 (1996)

DNA Vaccine


Gene Encoding Antigen
CMVintA
Promoter
Terminator
PolyA (BGH)
Bacterial
Selection Gene

Generation of CTL by DNA Vaccines



1918 Flu Pandemic

20 Million Deaths

Courtesy of T Sharrar, Smithsonian Institution

Initial Demonstration of Efficacy of DNA Vaccines

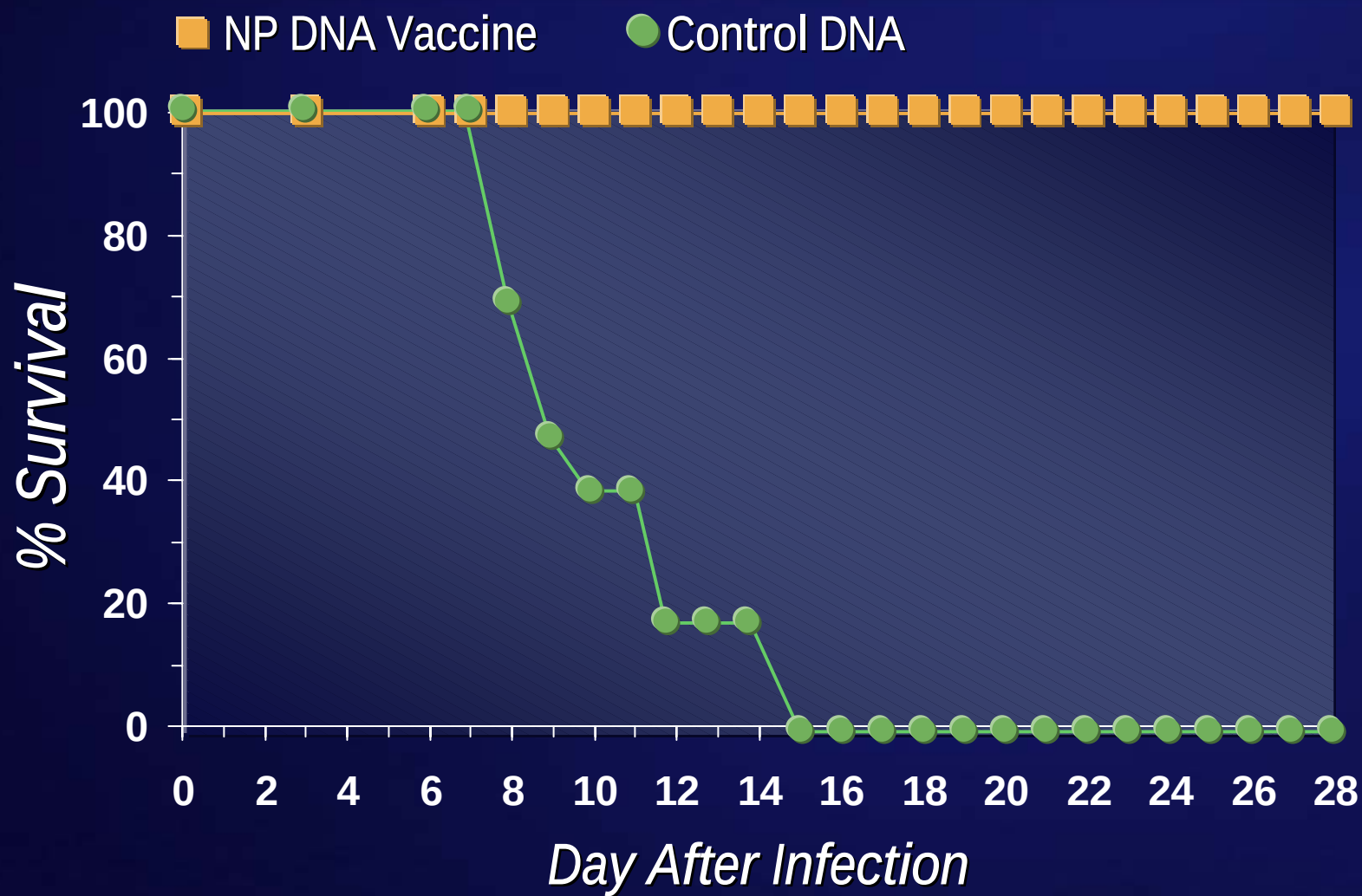
- Generation of CTL by DNA vaccine
- Protection by DNA vaccine against infectious challenge
- Cross-strain protection

H1N1 *(1934)*

H3N2 *(1968)*

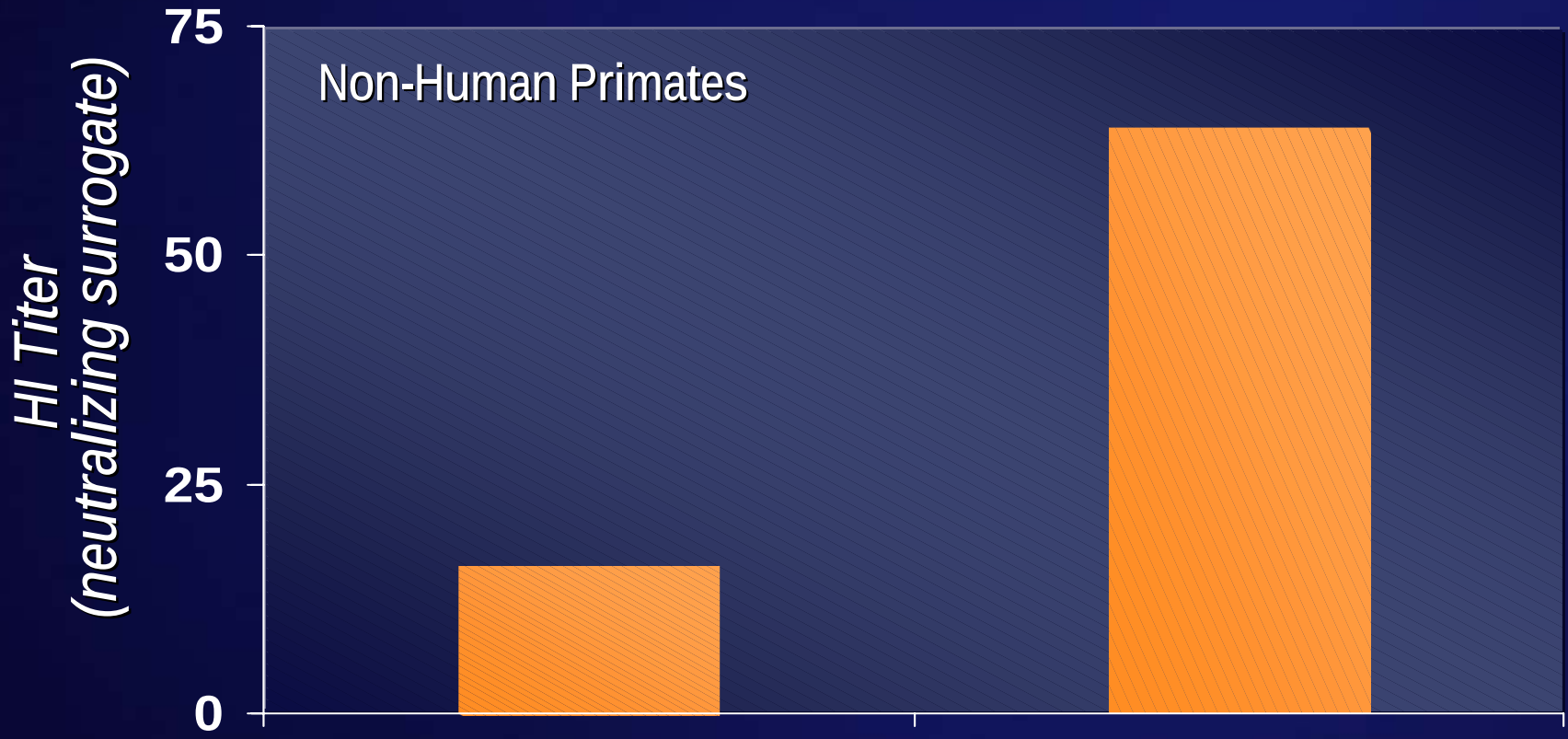
Ulmer JB, Donnelly JJ…Liu MA, Science 259: 1745 (1993)

DNA Vaccine Protects Against Cross-Strain Influenza Challenge



Fu T-M…Liu MA and Donnelly JJ, J Virol 71:2715 (1997)

Addition of Irrelevant Plasmid DNA Increases Antigen-Specific Immune Responses

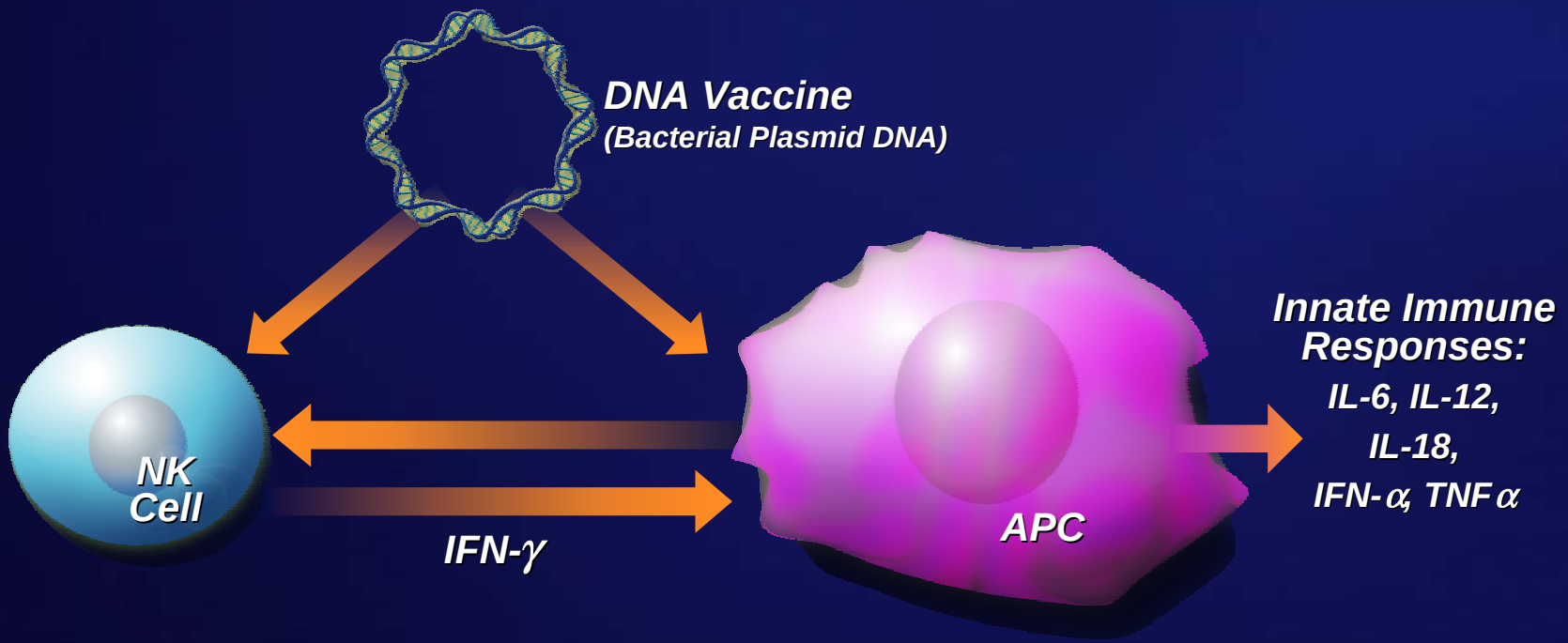


Donnelly JJ…Liu MA, Ann Rev Imm 15:627 (1997)

Immune Responses of DNA Vaccines

Results from:

- Specific immunity against encoded antigen
- Non-specific immune effects of plasmid backbone



Modified from Krieg, AM, Current Op Imm 12: 35 (2000)

Plasmid Non-Specific Stimulation

Due to:

- PuPuCGPyPy sequences
  - "CpG motifs"
- Potential means to increase / decrease / or change nature of immunogenicity of DNA Vaccines

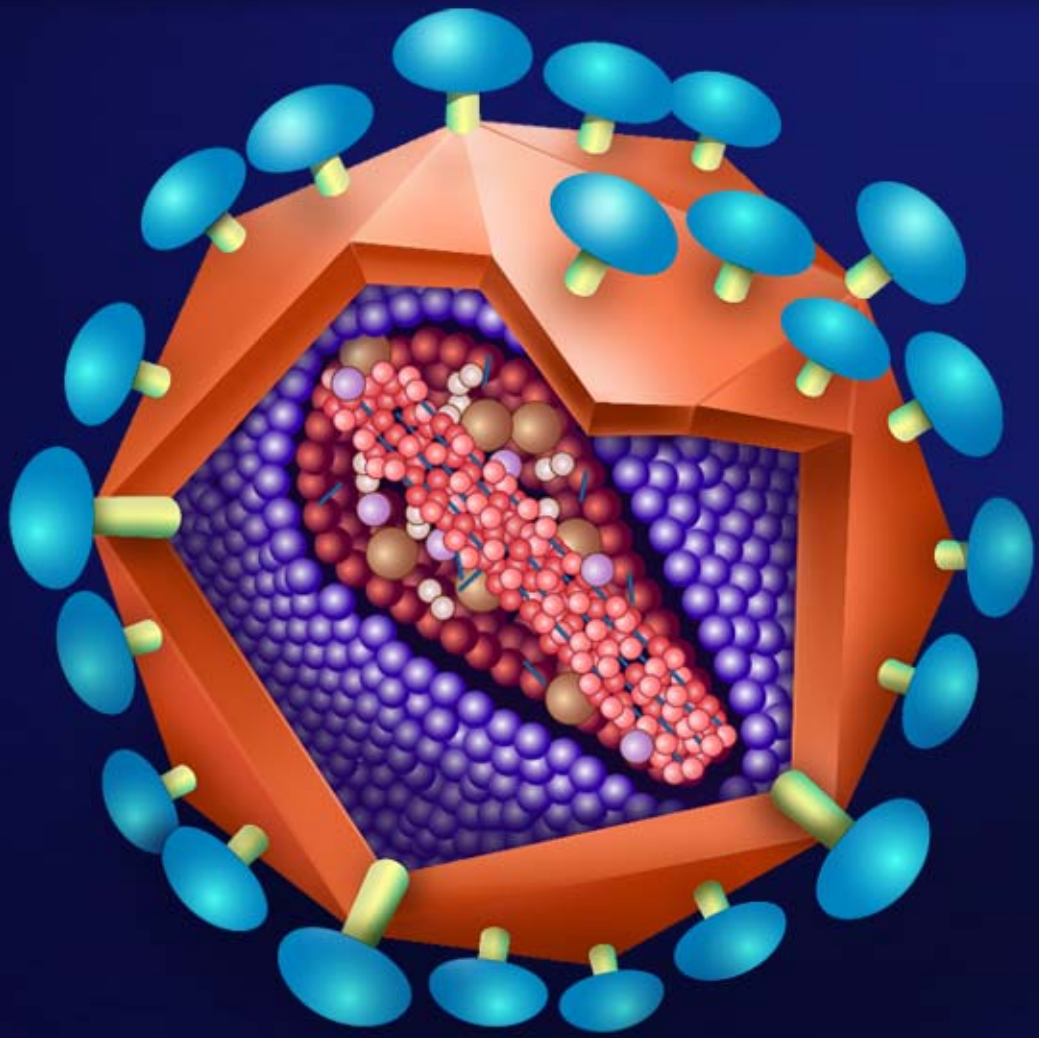
Krieg AM…Klinman DM, Nature 374:546 (1995)

Klinman DM…Krieg AM, PNAS 93:2879 (1996)

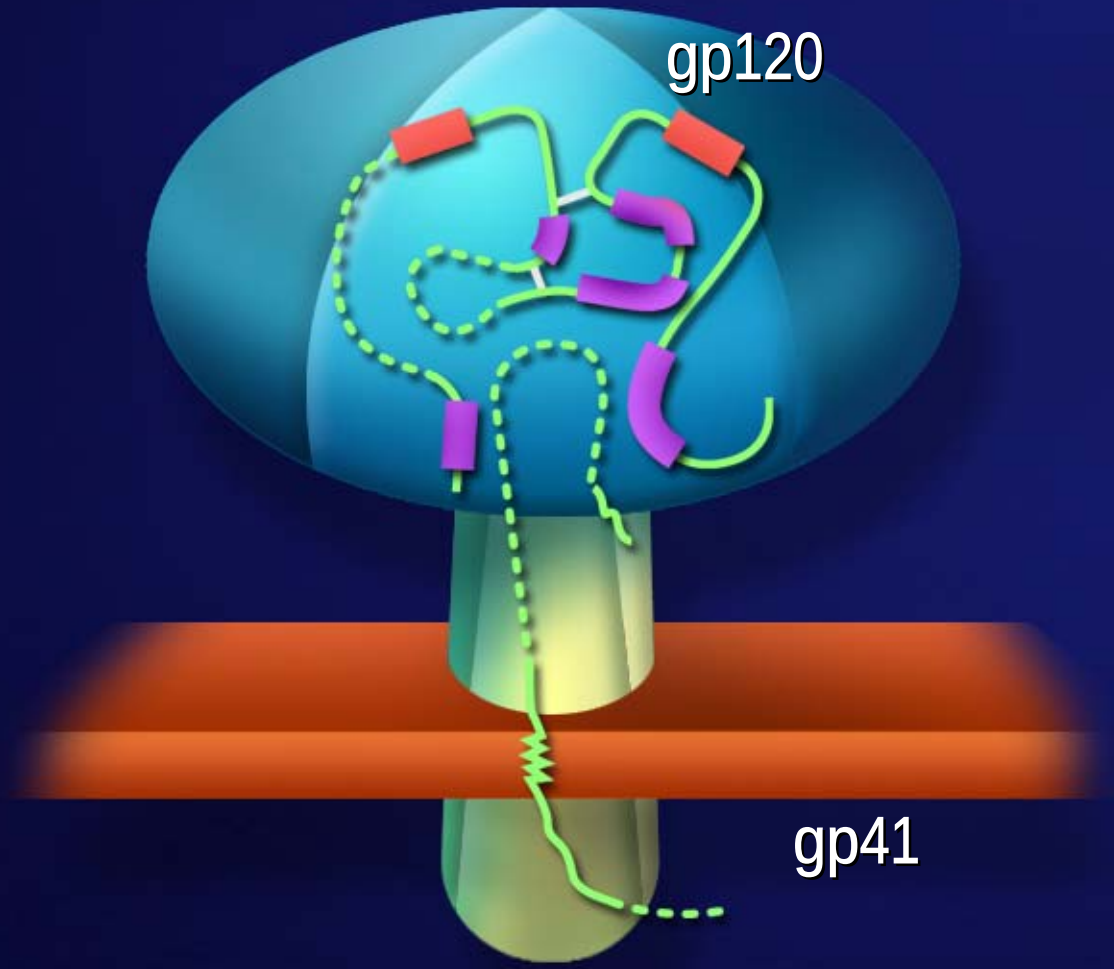
Sato Y…Carson DA and Raz E, Science 273:352 (1996)

Klinman DM…Ishijatsubo Y, JI 158:3635 (1997)

HIV



HIV Envelope



Different Forms of HIV Envelope Used for Immunizations

Monomer
gp120

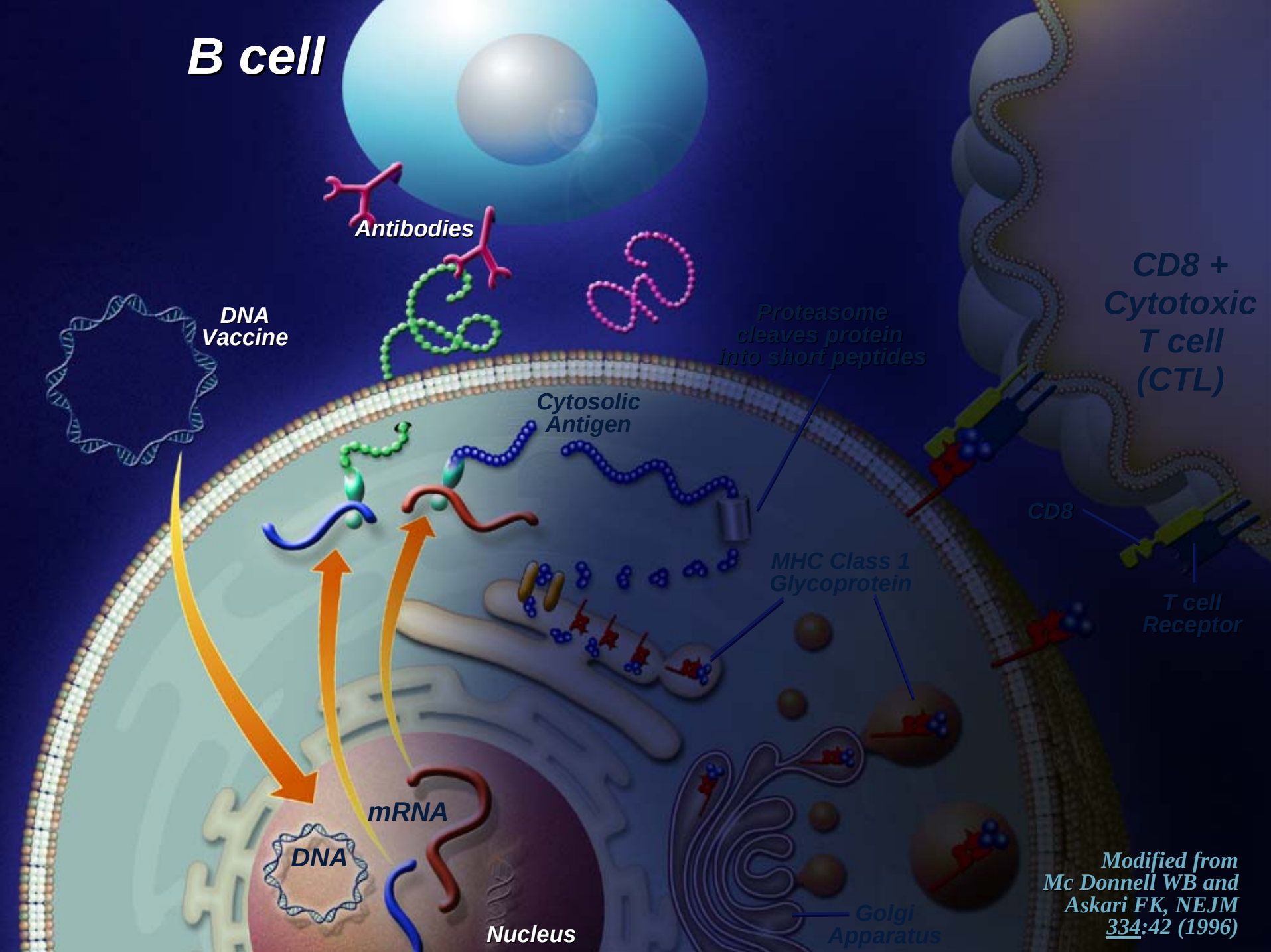
Recombinant
protein

Soluble Oligomer
gp140

Recombinant protein

Membrane Bound
gp160

DNA vaccine


B cell
Antibodies
DNA
Vaccine
Proteasome
cleaves protein
into short peptides
CD8 +
Cytotoxic
T cell
(CTL)
Cytosolic
Antigen
CD8
MHC Class 1
Glycoprotein
T cell
Receptor
mRNA
DNA
Nucleus
Golgi
Apparatus
Modified from
Mc Donnell WB and
Askari FK, NEJM
334:42 (1996)

Clinical Trials of DNA Vaccines

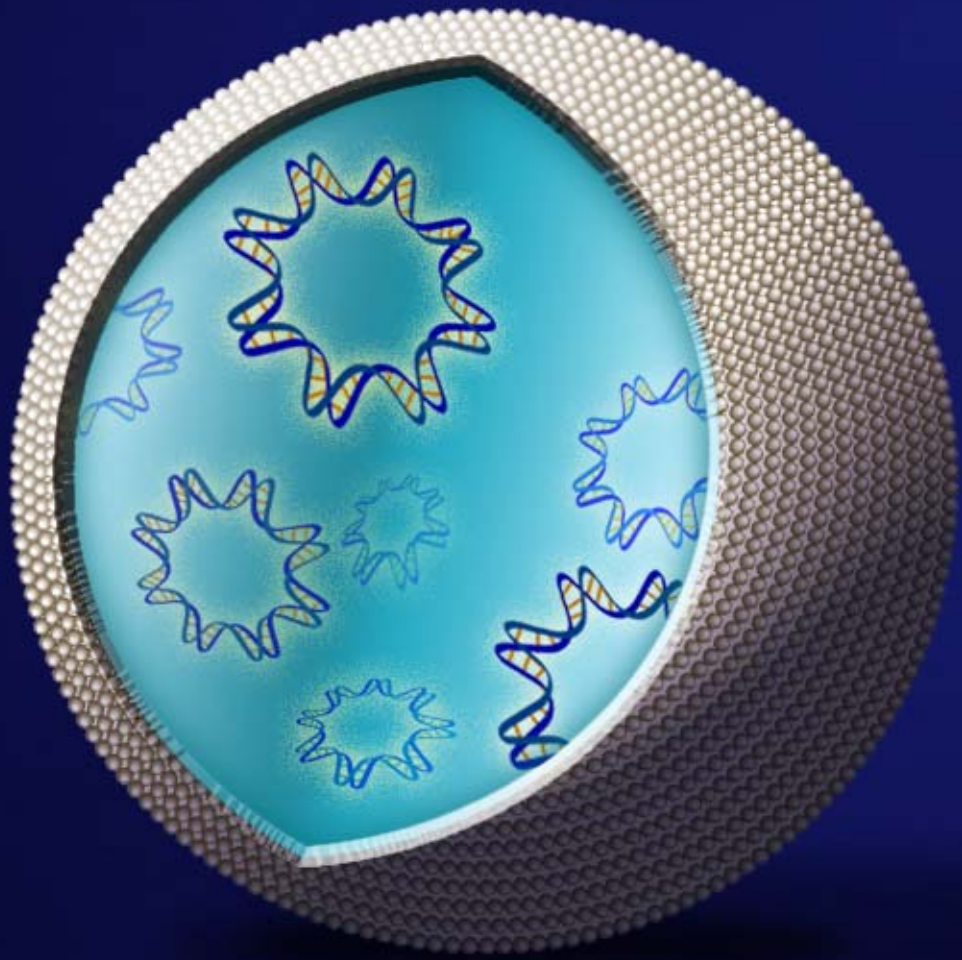
- HIV
  - Therapeutic and prophylactic
  - Multiple vaccines / multiple trials
- Influenza
- Malaria
  - Multiple vaccines / multiple trials
  - Antigen + cytokine genes
- Hepatitis B
- Cancer
- (Gene Therapy)

Second Generation DNA Vaccines

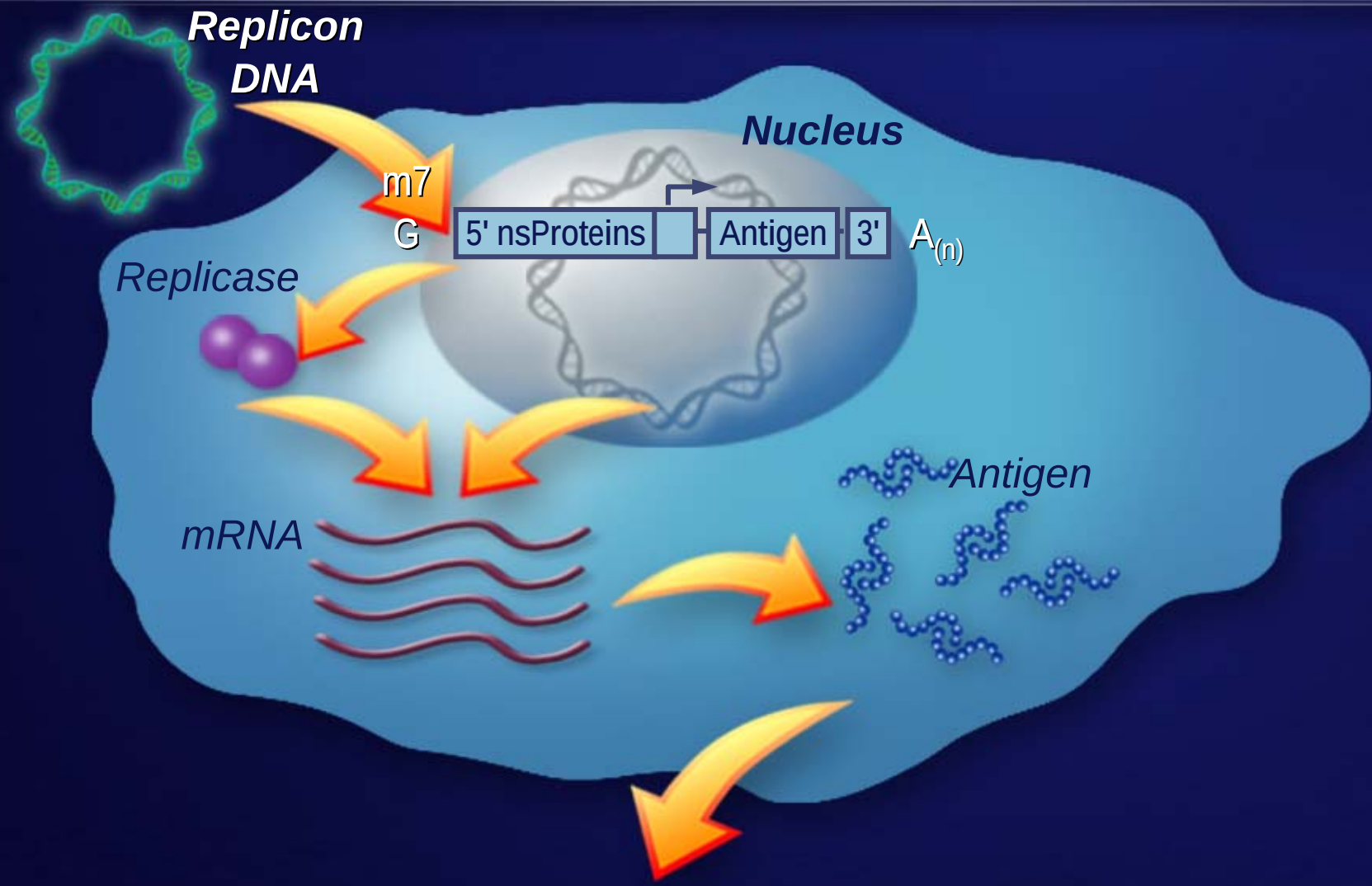
- Increased potency
- “Designer” immune response
- Oral delivery

Area of Mucosal Surfaces: 1½ Basketball Courts

Encapsulated DNA: Microparticles



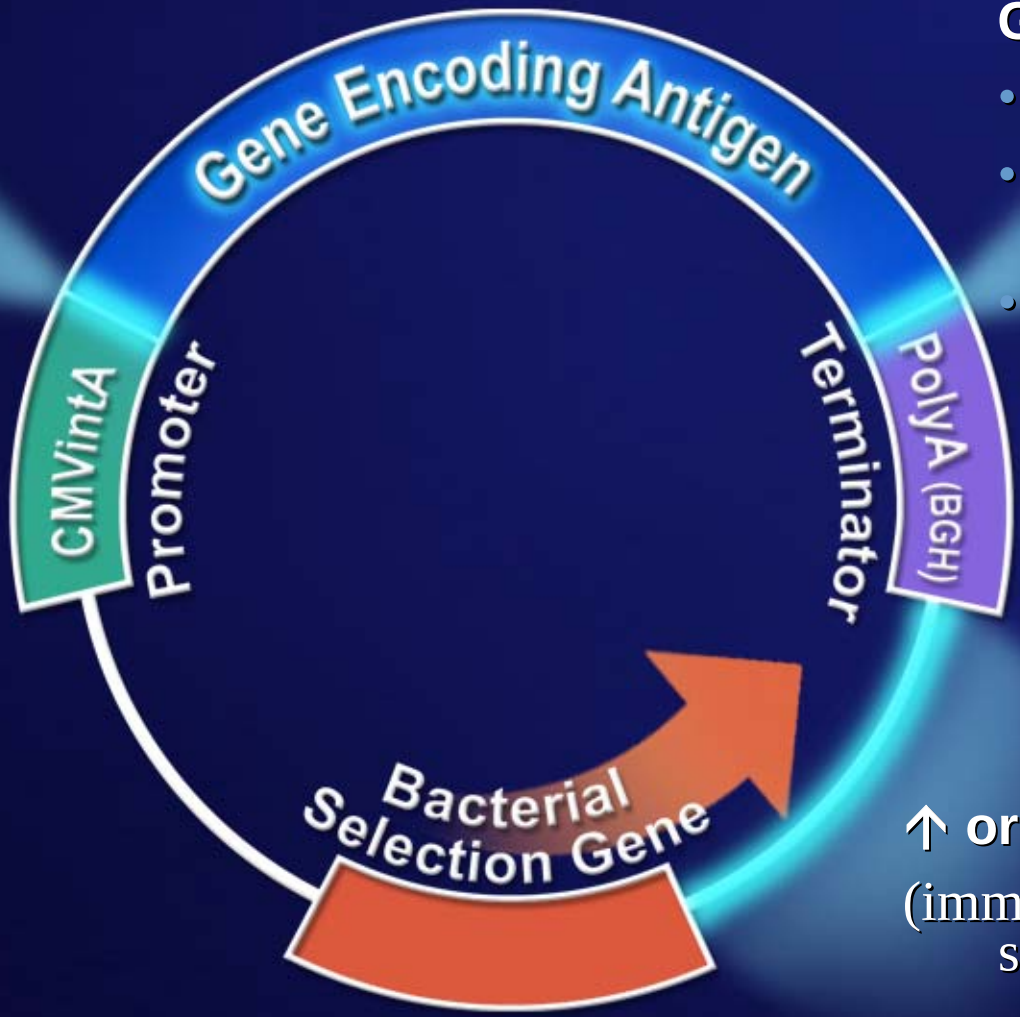
DNA Vaccine Replicons Rapidly Produce More Protein Antigen



“Designer Gene Vaccines”

Replicon:

- Amplify antigen mRNA



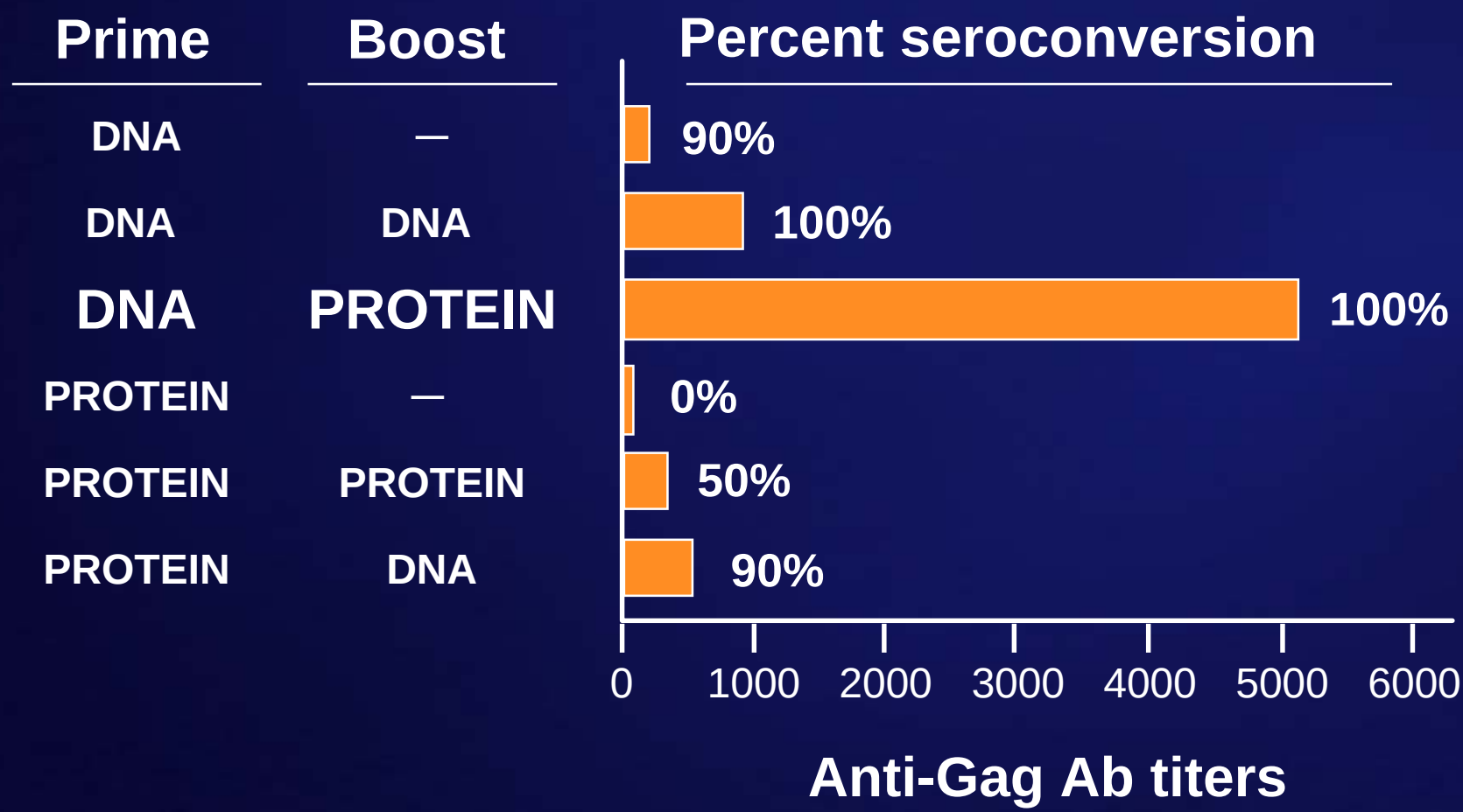
Genes Encoding:

- Cytokines
- Co-stimulatory molecules
- Targeting molecules

↑ or ↓ CpG Content:

(immunostimulatory sequences)

Sequential Immunization with DNA then Protein Generates Optimal Antibody Responses



Protection of BALB/c mice after immunization with plasmid DNA and/or recombinant MVA

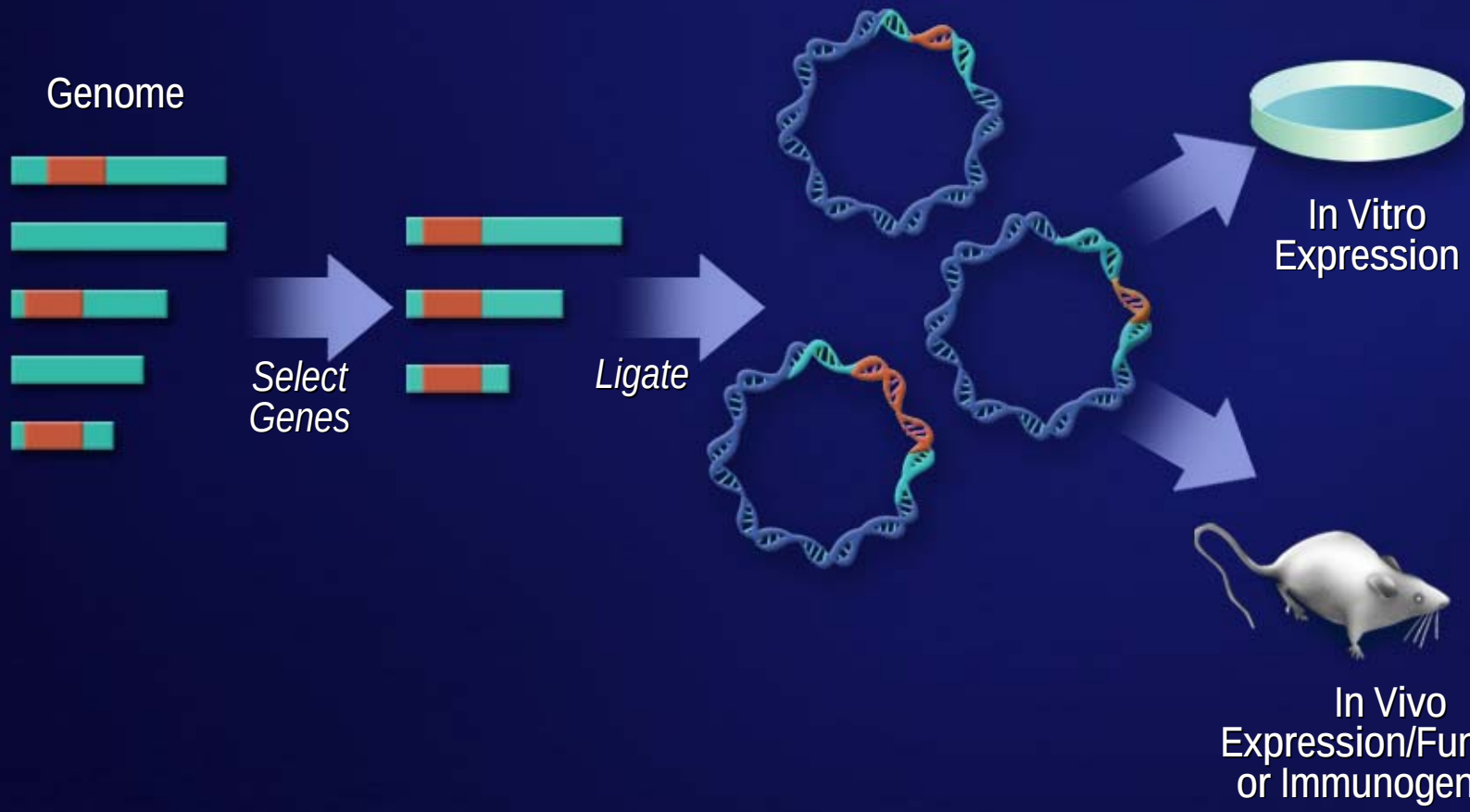
Immunization 1	Immunization 2	% Protection*
DNA	DNA	0
MVA	MVA	20
DNA	MVA	100
MVA	DNA	0

*5 animals/group

Antigens used: PbCSP + PbTRAP

J. Schneider, …, A.V.S. Hill, *Nature Medicine* 4:397-402

DNA Vaccines: Tool for Functional Genomics/Proteomics



Characteristic of DNA Vaccines

- Able to generate CTL, antibodies, T_H
  - Cross-strain protective CTL
  - Advantages of antigen structure for antibodies
    - Transmembrane protein
    - Native glycosylation
  - T_H intrinsically T_H 1
    - Can co-deliver cytokines to augment or alter T_H phenotypes
  - Mechanisms for CTL and T_H generation elucidated
  - Ability to stimulate desired immune responses not induced by wild-type disease
  - Avoid certain limitations/concerns of viral vectors

Characteristics of DNA Vaccines

- Second generation DNA Vaccines
  - Increased potency
  - Oral/Mucosal delivery
  - Facile manipulation of immune responses
- Potential advantages for clinical usage
  - Ability to generate T cell immunity: critical for many unconquered diseases
  - Key characteristics relevant to globally-needed vaccines
    - Generic technology
    - Stability
    - Manufacturing ease
    - Cost
    - Potential duration of immune response

Disease Models in Which DNA Vaccines Have Demonstrated Efficacy

Infectious Diseases

Viruses

- HIV
- Influenza
- Rabies
- Hepatitis B,C,D
- Ebola
- Herpes Simplex
- Papilloma
- CMV
- Rota
- Measles
- LCMV
- St. Louis Enceph

Bacteria

- B. Burgdorferi
- C. tetani
- M. Tb
- S. typhi

Parasites/Protozoa

- Malaria
- Mycoplasma
- Leishmania
- Schistosoma
- Taenia ovis
- Toxo. gondii

Cancer

- Breast (Her2/neu)
- Colon
- Prostate
- Myeloma
- Lymphoma
- E7-Induced
- Fibrosarcoma

Allergy

- House Dust Mite
- Peanut
- Experimental Airway Hyperresponsiveness

Autoimmune Disease

- Diabetes
- EAE (MS model)