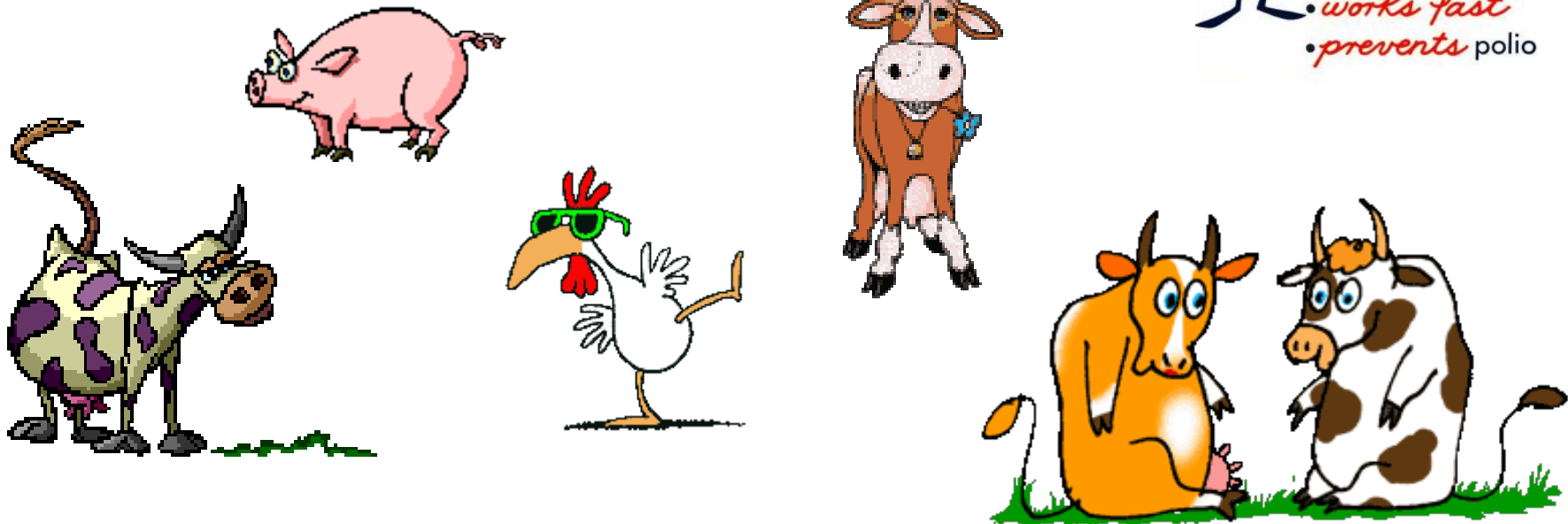


VIF Immunology and Vaccines course

Gregers Jungersen, DVM, Ph.D,
Professor in Adaptive Immunology and Vaccinology



Today's program

Immunology and vaccines recap

Immunologic correlates of protection

The right vaccine for the right infection

Vaccines for us and our children

New developments

Vaccine debate

Vaccination issues for production animals/examples

I need two voluntary feed-back persons

The course of an infection

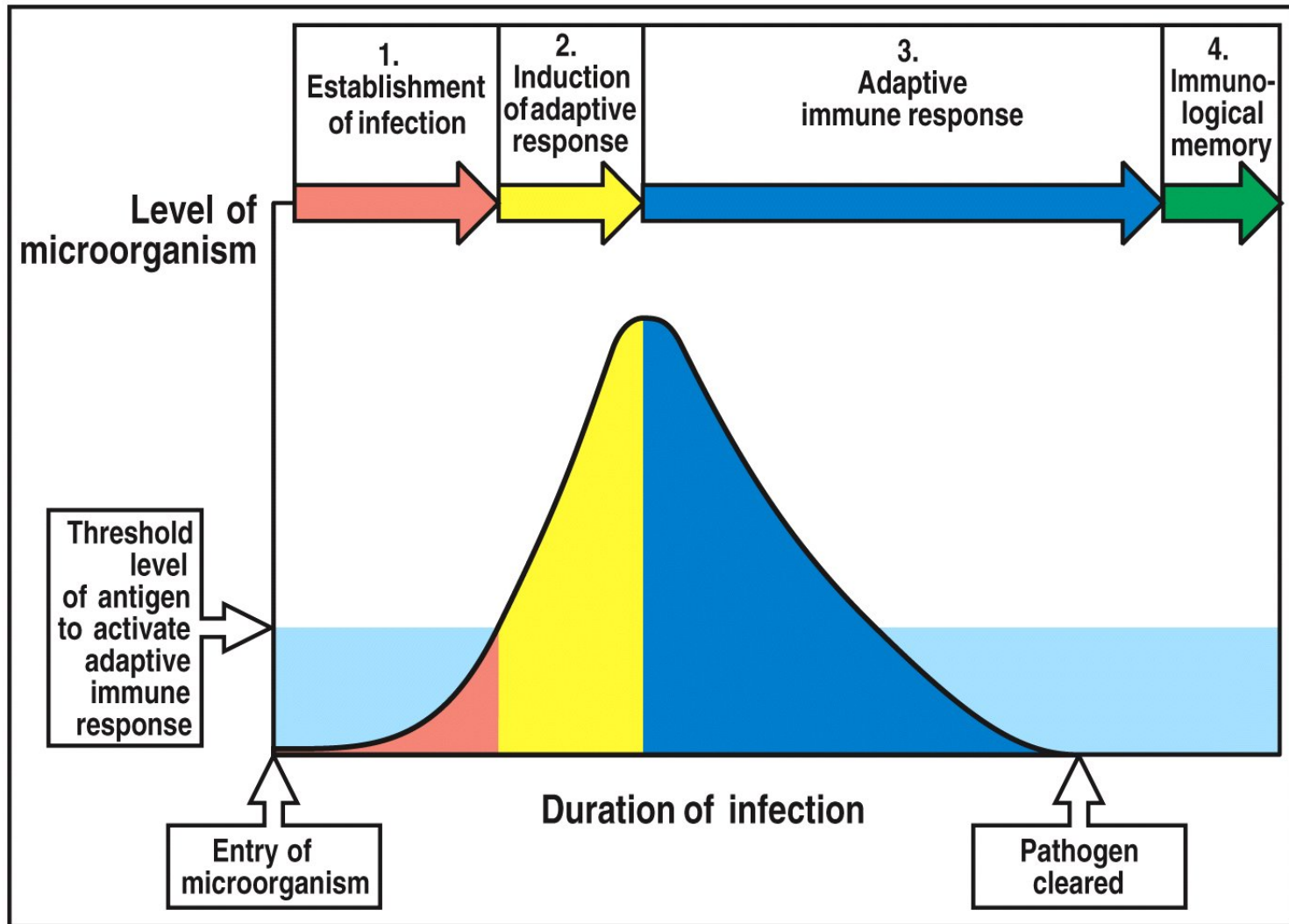


Figure 10-1 Immunobiology, 6/e. (© Garland Science 2005)

Response to VACCINATION

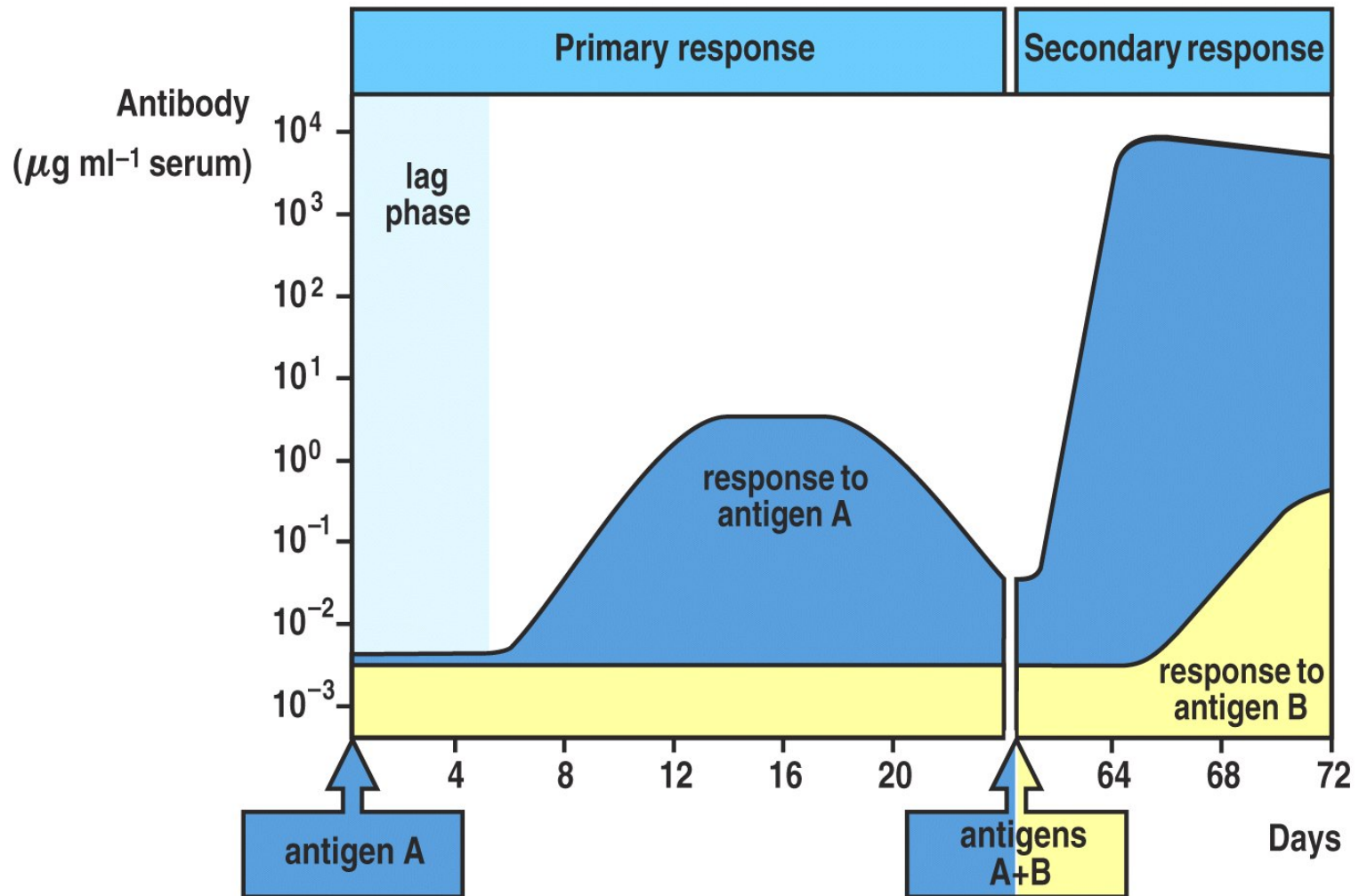


Figure 1-20 Immunobiology, 6/e. (© Garland Science 2005)

**The immune system protects against 4 types of pathogens
- each type is dealt with differently**

Type of pathogen	Example	Disease	Relevant immune response
Extracellular bacteria, fungi and parasites	<i>Streptococcus pneumoniae</i> <i>Clostridium tetani</i> <i>Trypanosoma brucei</i> <i>Actinobaccilus pleuropneumoniae</i>	Pneumoniae (pneumococci) Tetanus Trypanosomiasis (sleeping sickness) Porcine pleuropneumoniae	ANTIBODIES against toxins or surface antigens
Intracellular bacteria and parasites	<i>Mycobacterium leprae</i> <i>Mycobacterium tuberculosis</i> <i>Plasmodium falciparum</i> <i>Toxoplasma gondii</i>	Leprosy Tuberculosis Malaria Toxoplasmosis (zoonosis)	ANTIBODIES against extracellular stages HELPER T CELL activation of macrophages
Virus (intracellular)	Variola virus Influenza virus Varicella zoster virus	Smallpox Influenza Chicken-pox (children)	ANTIBODIES against extracellular stages KILLER T CELLS against infected cells
Parasitic helminths (extracellular)	<i>Ascaris</i> <i>Schistosoma spp.</i>	Ascariasis Bilharziosis (Schistosomiasis)	Eosinophil granulocytes Antibody dependent cell-mediated cytotoxicity (ADCC)

The innate immune response

- Phagocytic cells
 - Macrophages
 - Neutrophil granulocytes
- Inflammation
 - Interferon alpha (α) and beta (β)
 - Cytokines and chemokines
 - Complement system
 - Increased vascular permeability and attraction of cells
- NK cells

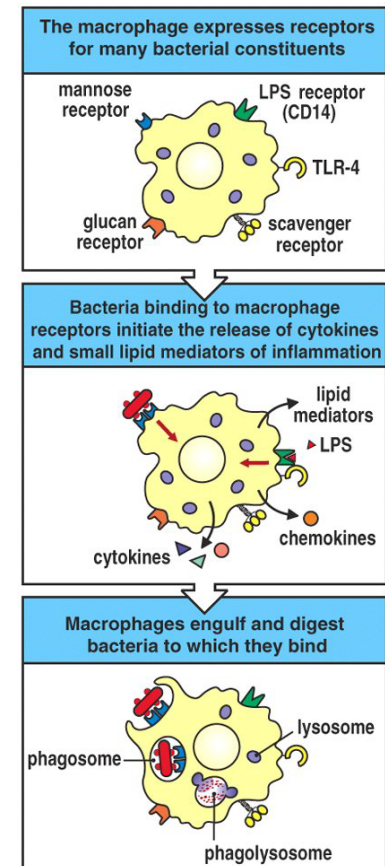
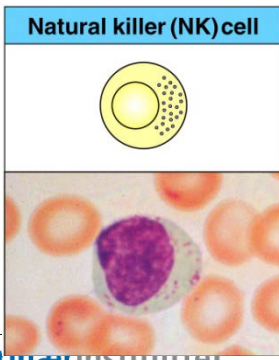


Figure 2-5 Immunobiology, 6/e. © Garland Sci

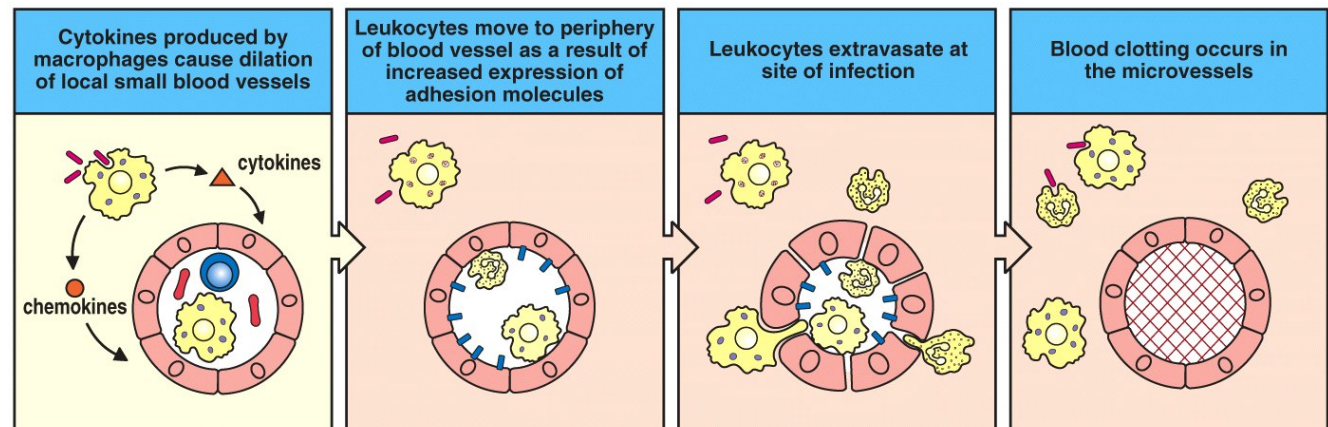


Figure 2-8 Immunobiology, 6/e. © Garland Science 2005



The adaptive immune response

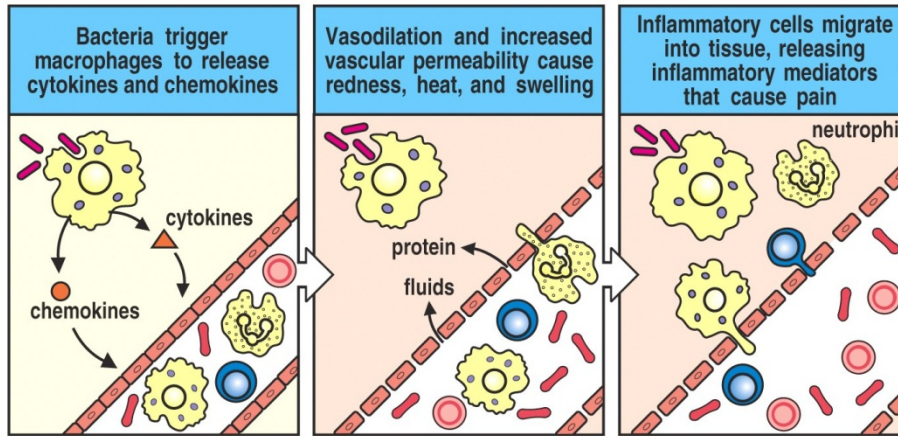
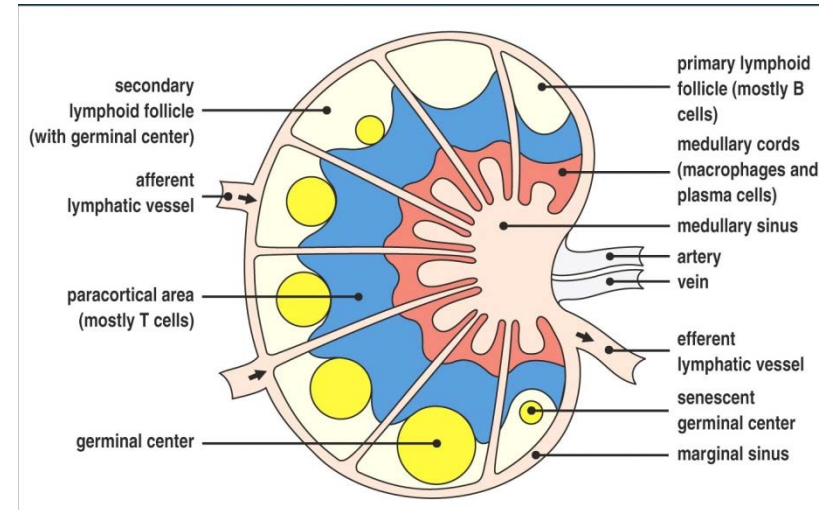


Figure 1-12 Immunobiology, 6/e. (© Garland Science 2005)

Local inflammation



Dendritic cells are activated

Heat

Redness

Swelling

Pain

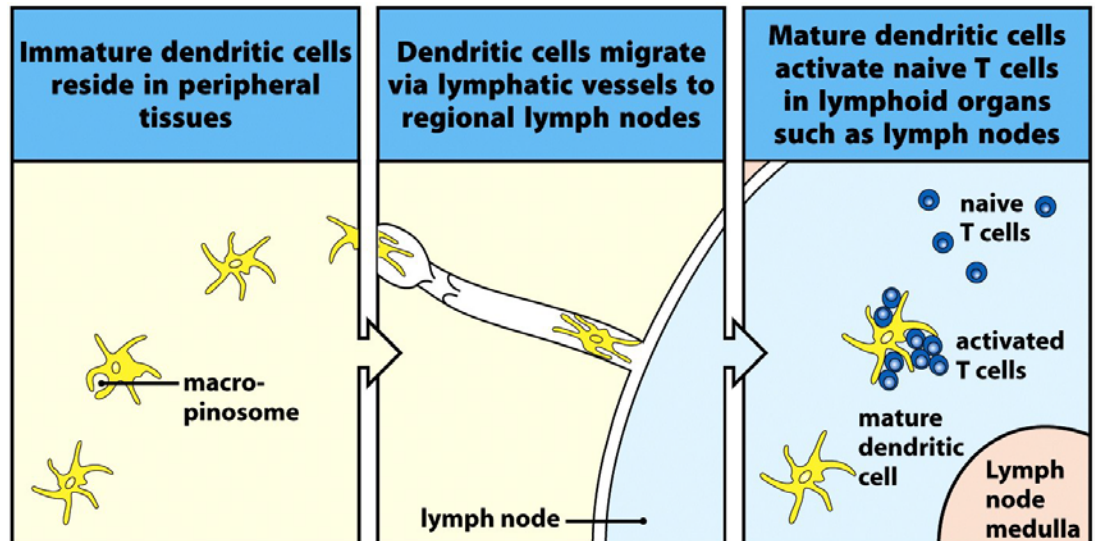


Figure 1-9 Immunobiology, 7ed. (© Garland Science 2008)

Only naive immune cells recognising the antigen will become activated

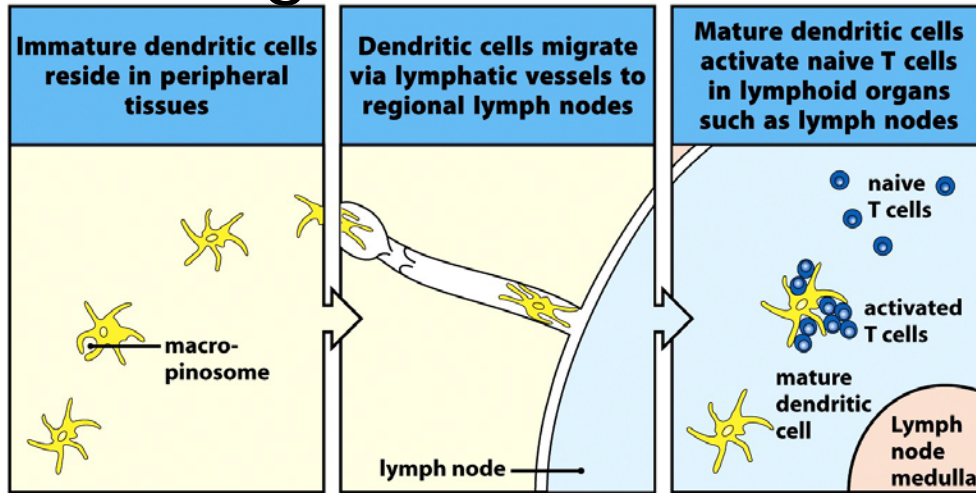
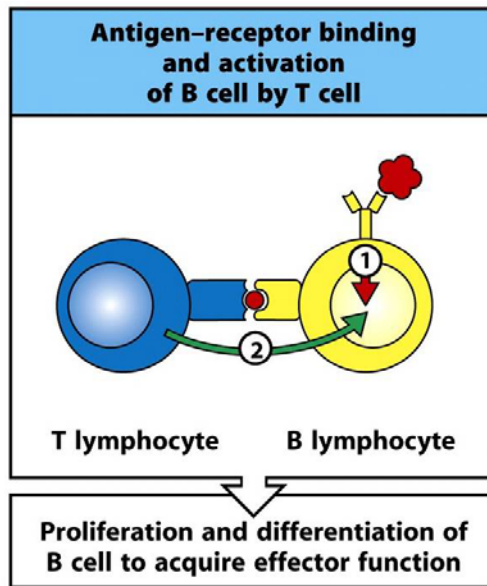
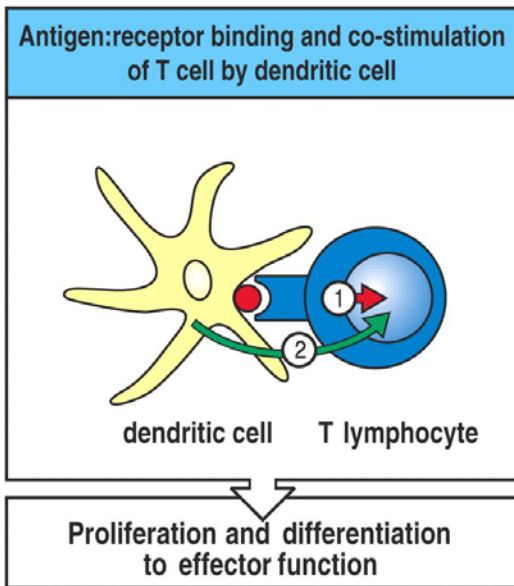
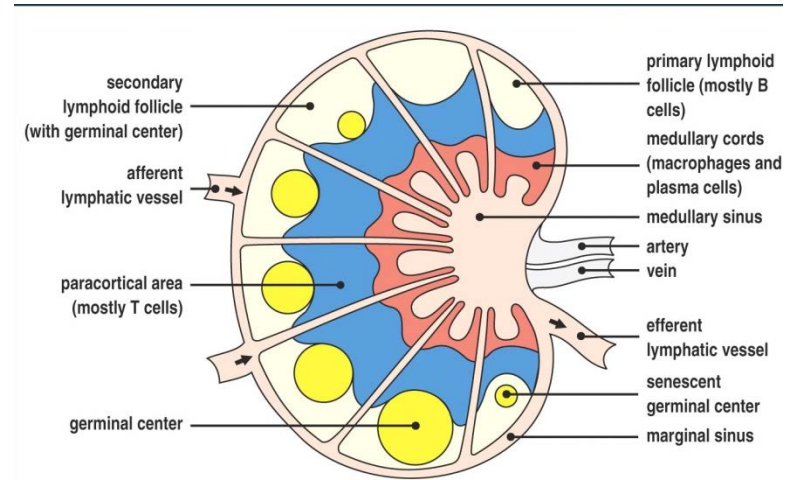


Figure 1-9 Immunobiology, 7ed. (© Garland Science 2008)



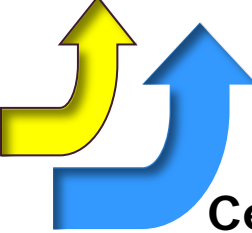

Cell division
 =
Proliferation
 =
Clonal expansion

Figure 1-21 Immunobiology, 6/e. (© Garland Science 2005)

Only lymphocytes with a T cell receptor specific for the antigen will become activated

Clonal selection

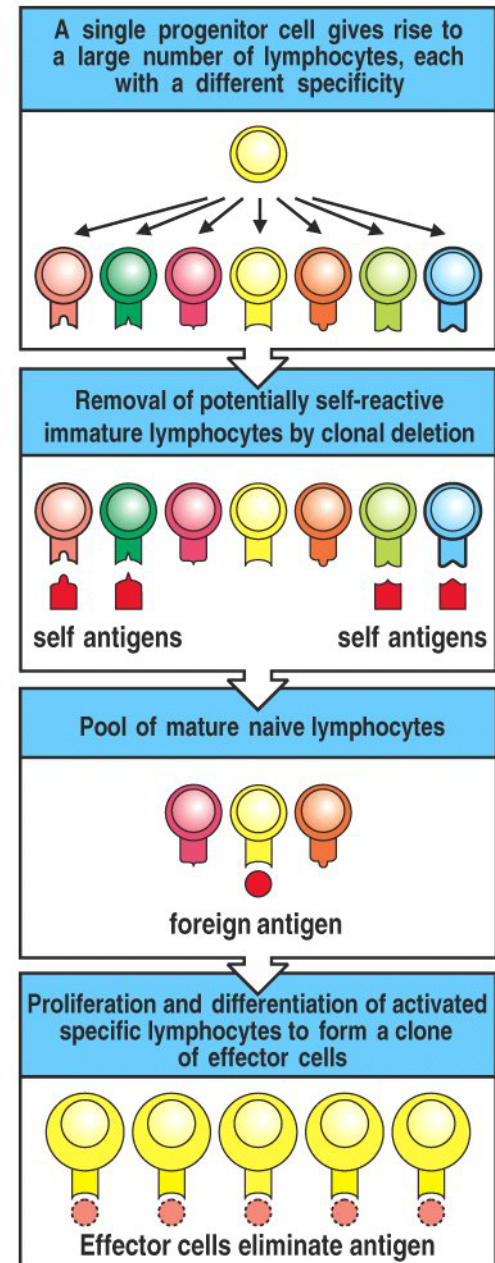


Figure 1-14 Immunobiology, 6/e. (© Garla

Affinity maturation of antibodies

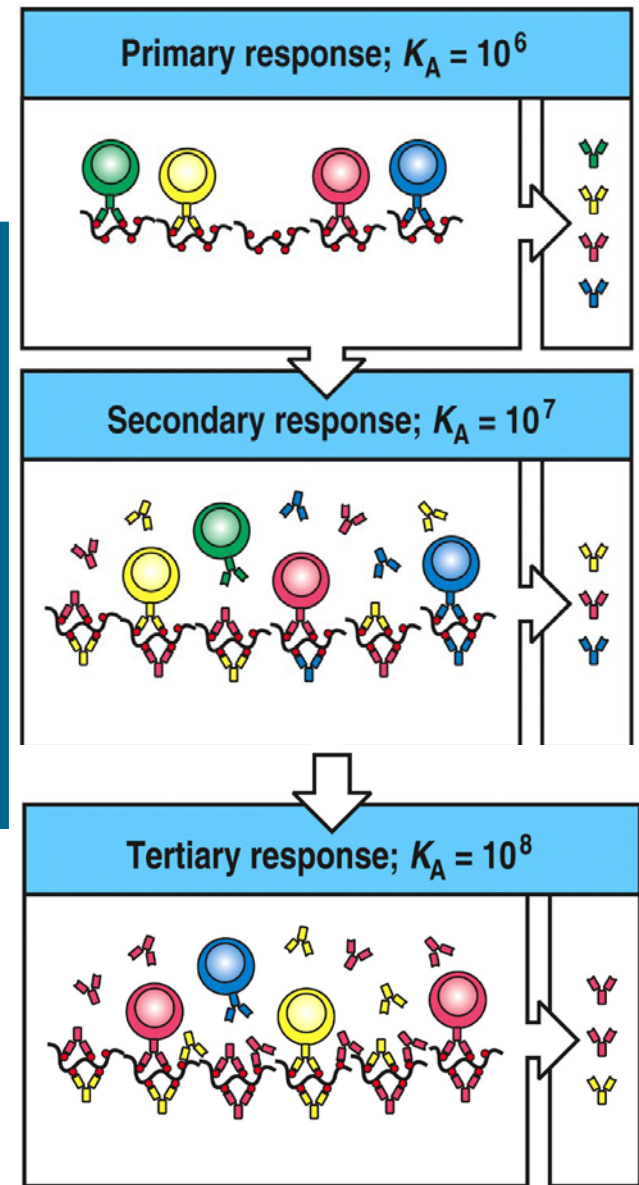
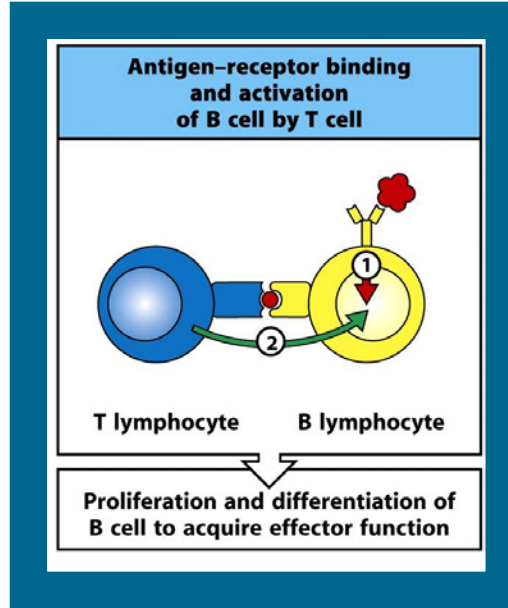
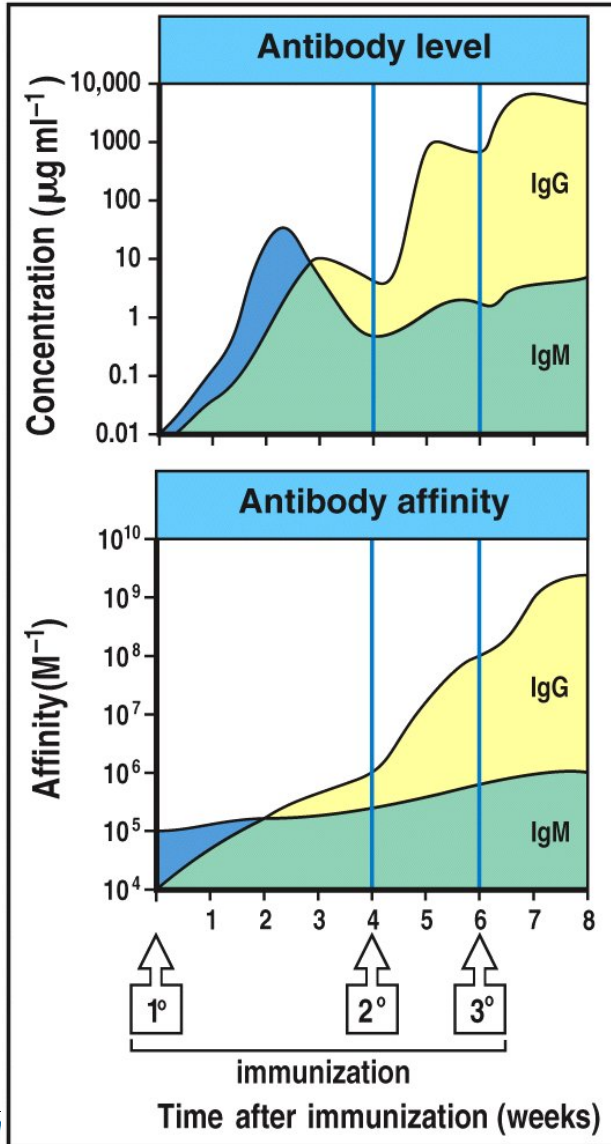
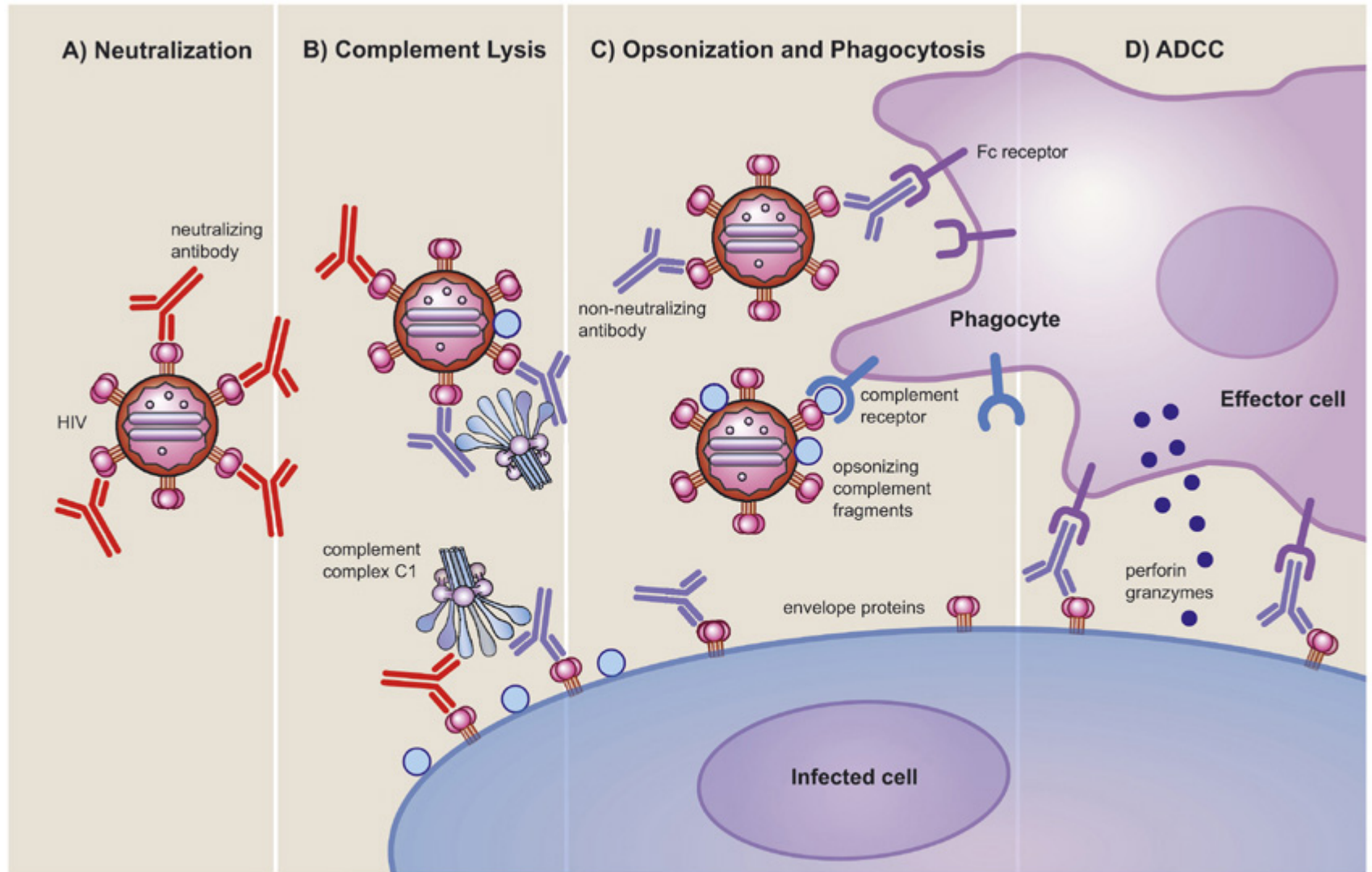


Figure 10-32 part 2 of 2. Immunobiology, 6/e. (© Garland Science 2005)

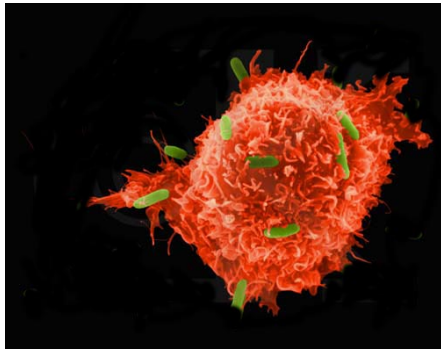
Effector mechanisms of antibodies



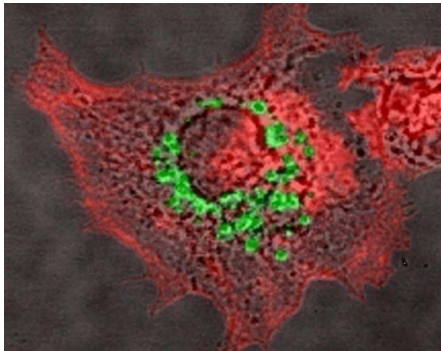
- proteins
- = neutralizing antibody
- ≡ non-neutralizing antibody
- opsonizing complement fragments
- complement complex C1
- envelope proteins
- perforin granzymes
- Fc receptor
- complement receptor

Complex interactions in the immune system

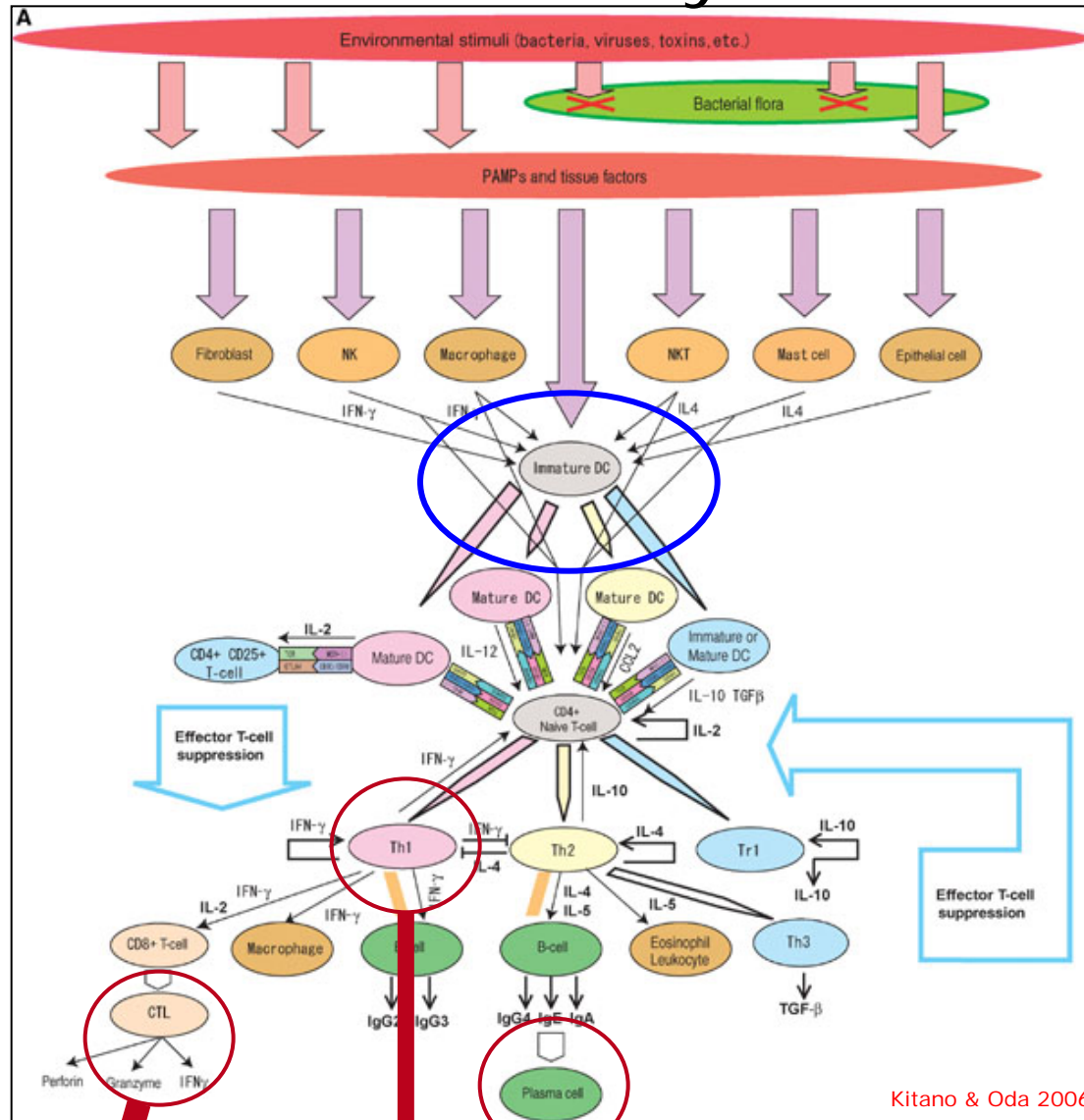
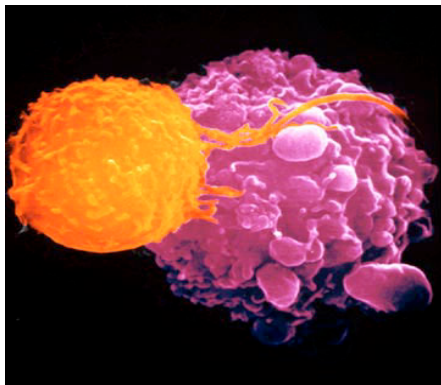
1. Contact



2. Cellular uptake



3. Interaction with immune cells



Kitano & Oda 2006

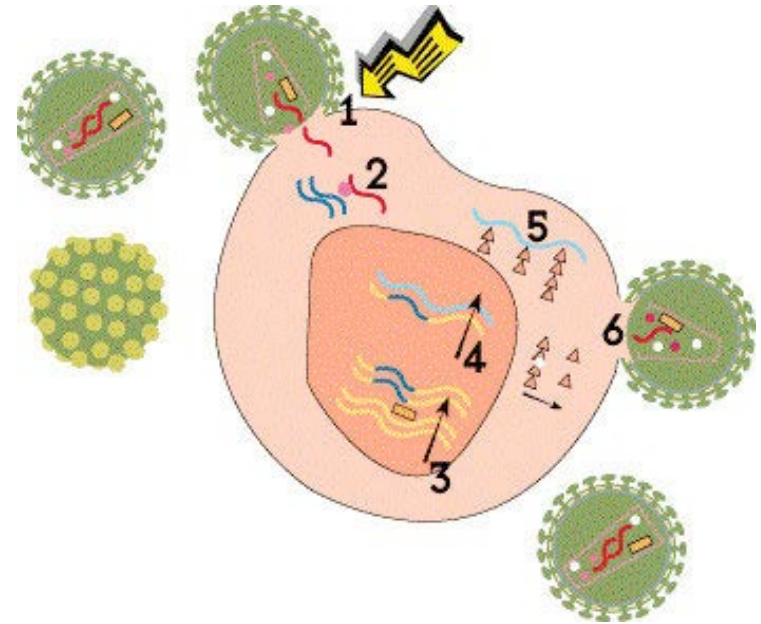
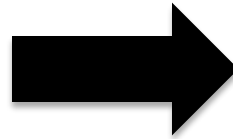
Virus Intracellular bacteria Extracellular bacteria/virus

Virus infection

Infection

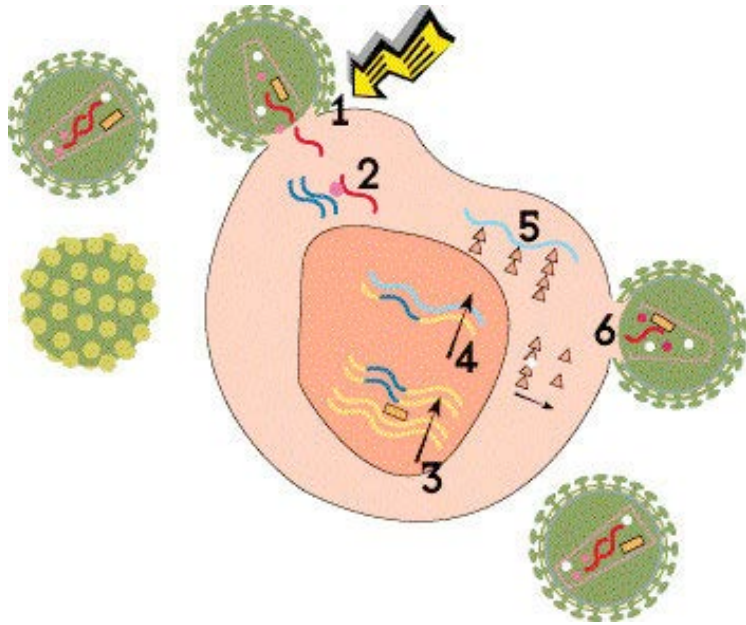


Virus exploits the host cells as factory for new virus particles



Virus infection

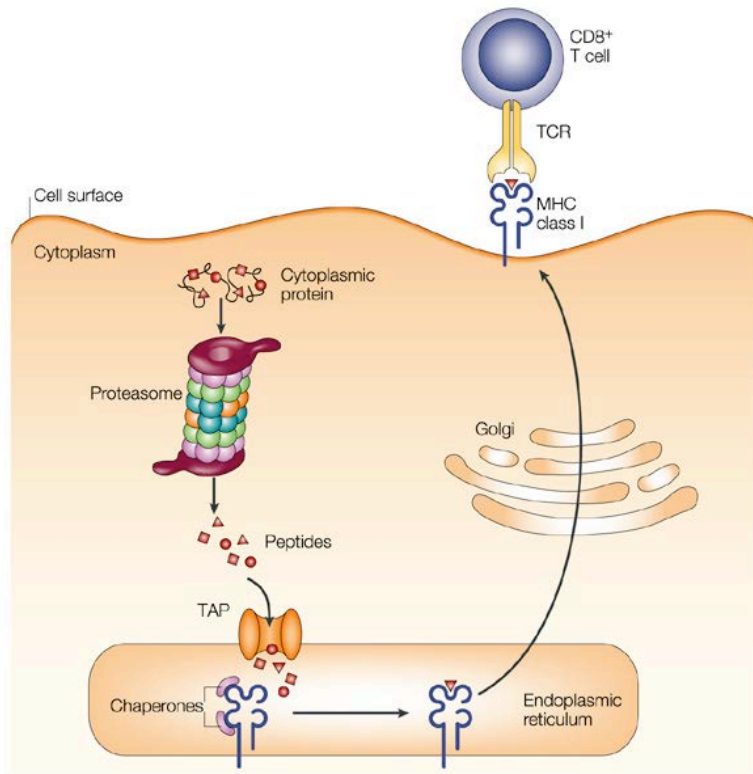
Virus exploits the host cells as factory for new virus particles



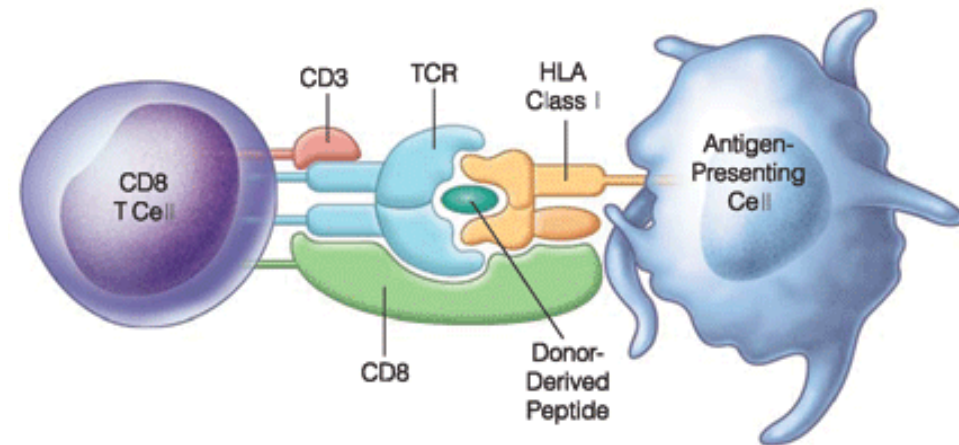
Antibodies can neutralize spreading to new cells,

But can't stop replication of virus

Peptides from "production" are loaded in MHC class I molecules



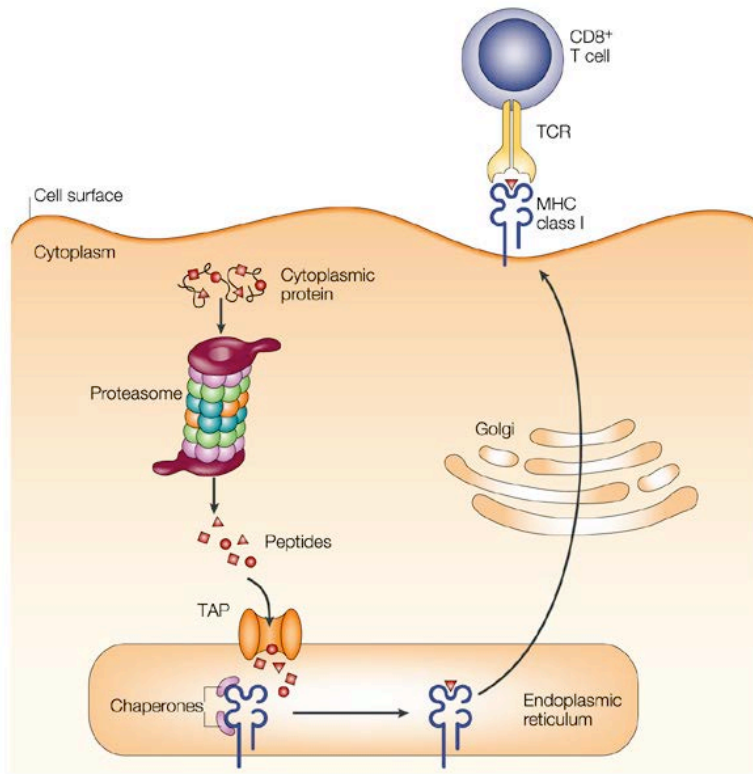
Nature Reviews | Immunology



Peptides from own and virus proteins are surface displayed in MHC class I complex

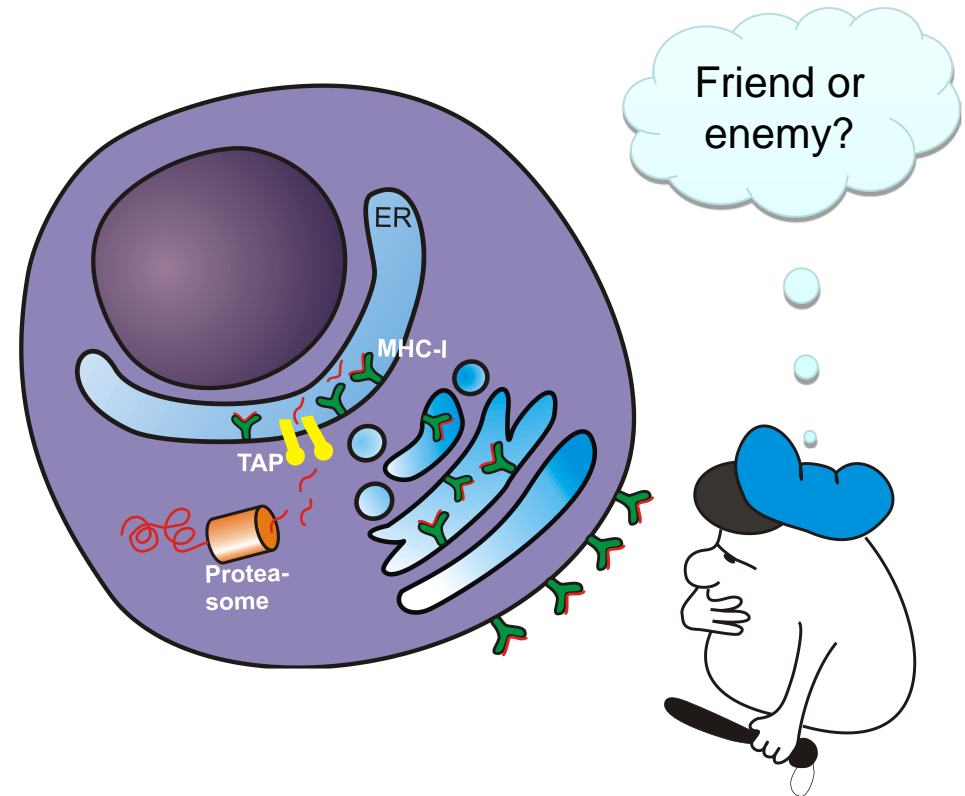
Cytotoxic T cells surveill cell-production

Peptides from "production" are loaded in MHC class I molecules



Nature Reviews | Immunology

Peptides from own and virus proteins are surface displayed in MHC class I complex



Cytotoxic T cells surveill cell-production

Virusinfected cell is killed by cytotoxic T cells

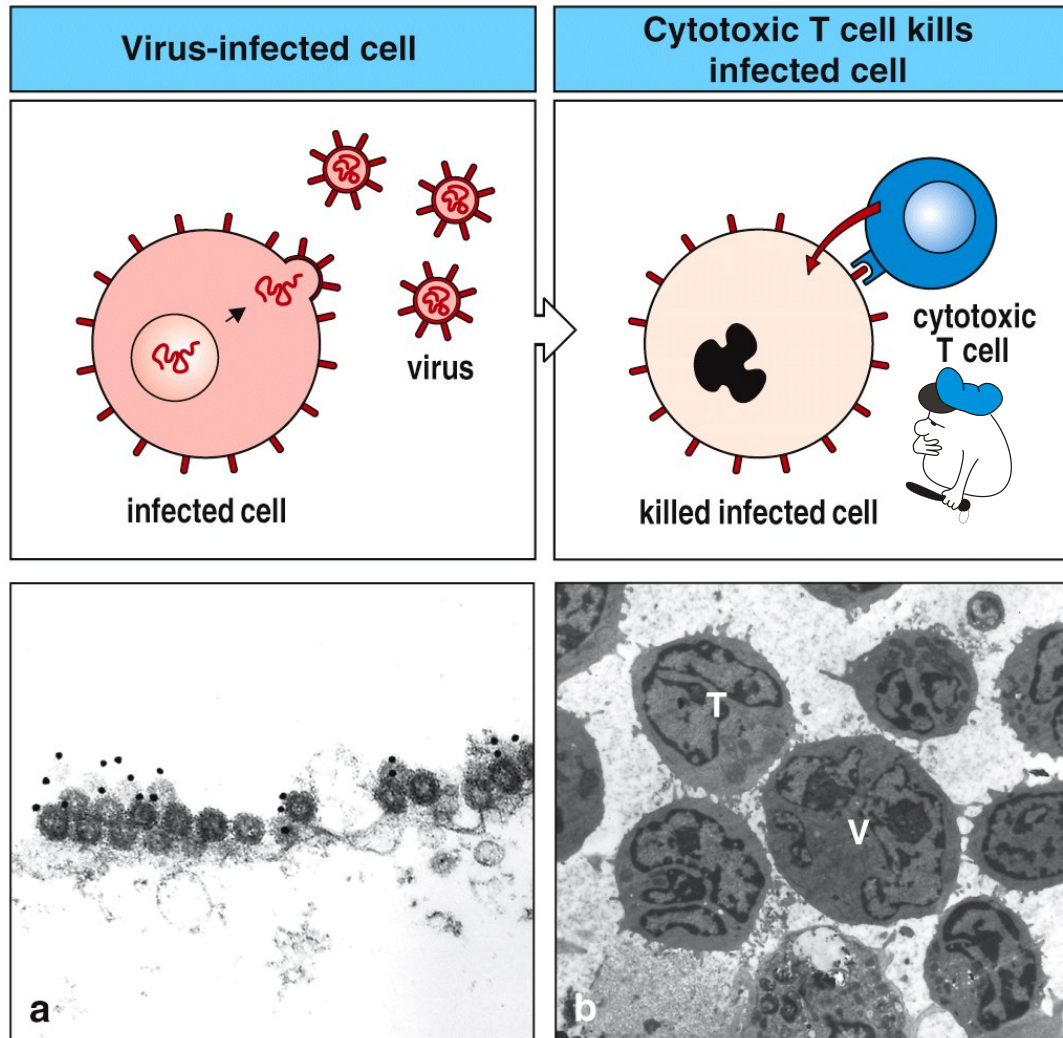
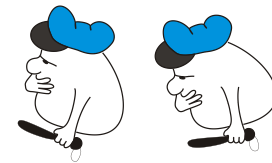


Figure 1-25 Immunobiology, 6/e. (© Garland Science 2005)

How do we measure virus-specific



after infection or vaccination?

Intracellular bacteria

Immunity depending on T helper (T_H1) cells and interferon-gamma ($IFN-\gamma$)

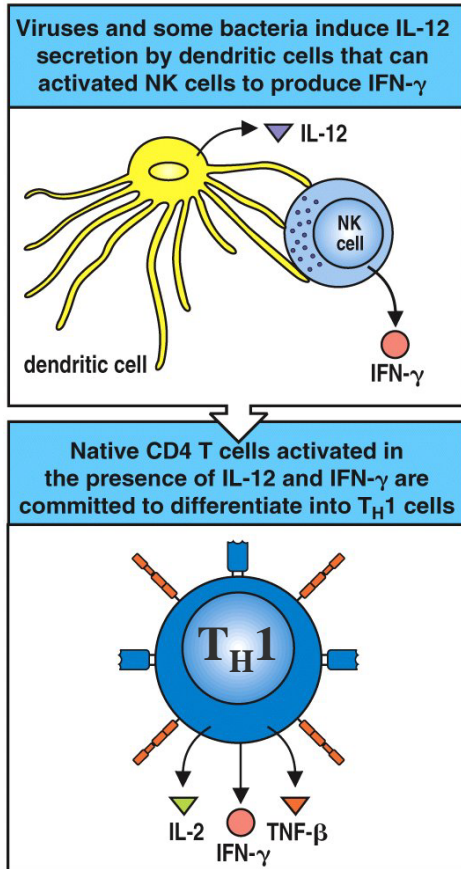


Figure 10-9 Immunobiology, 6/e. © Garland Science 2001.

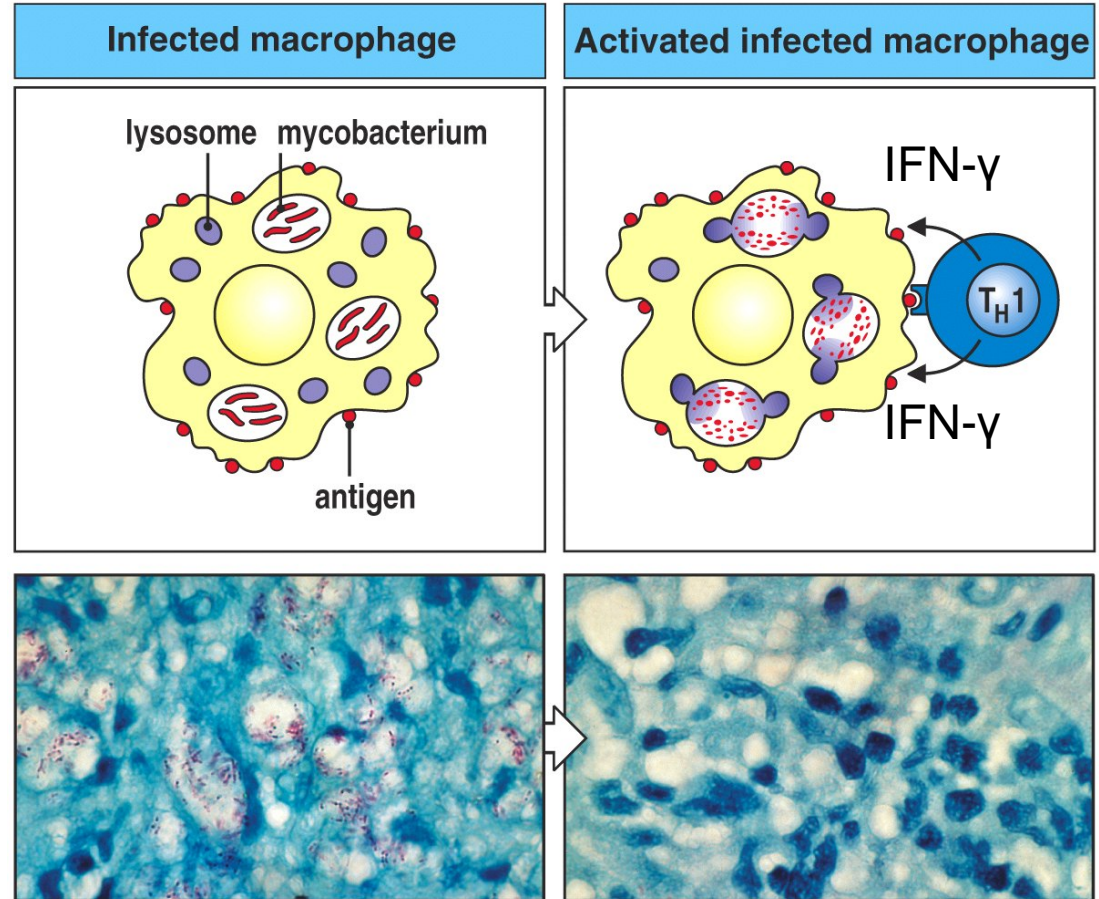
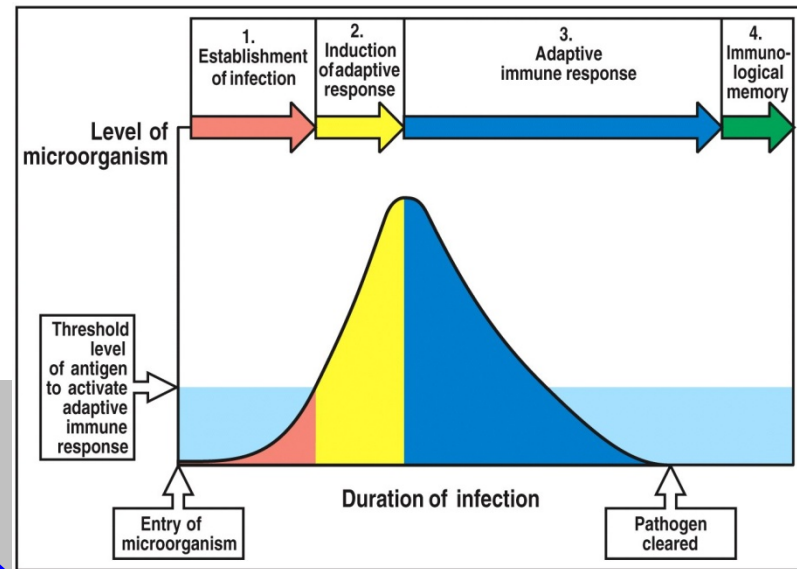
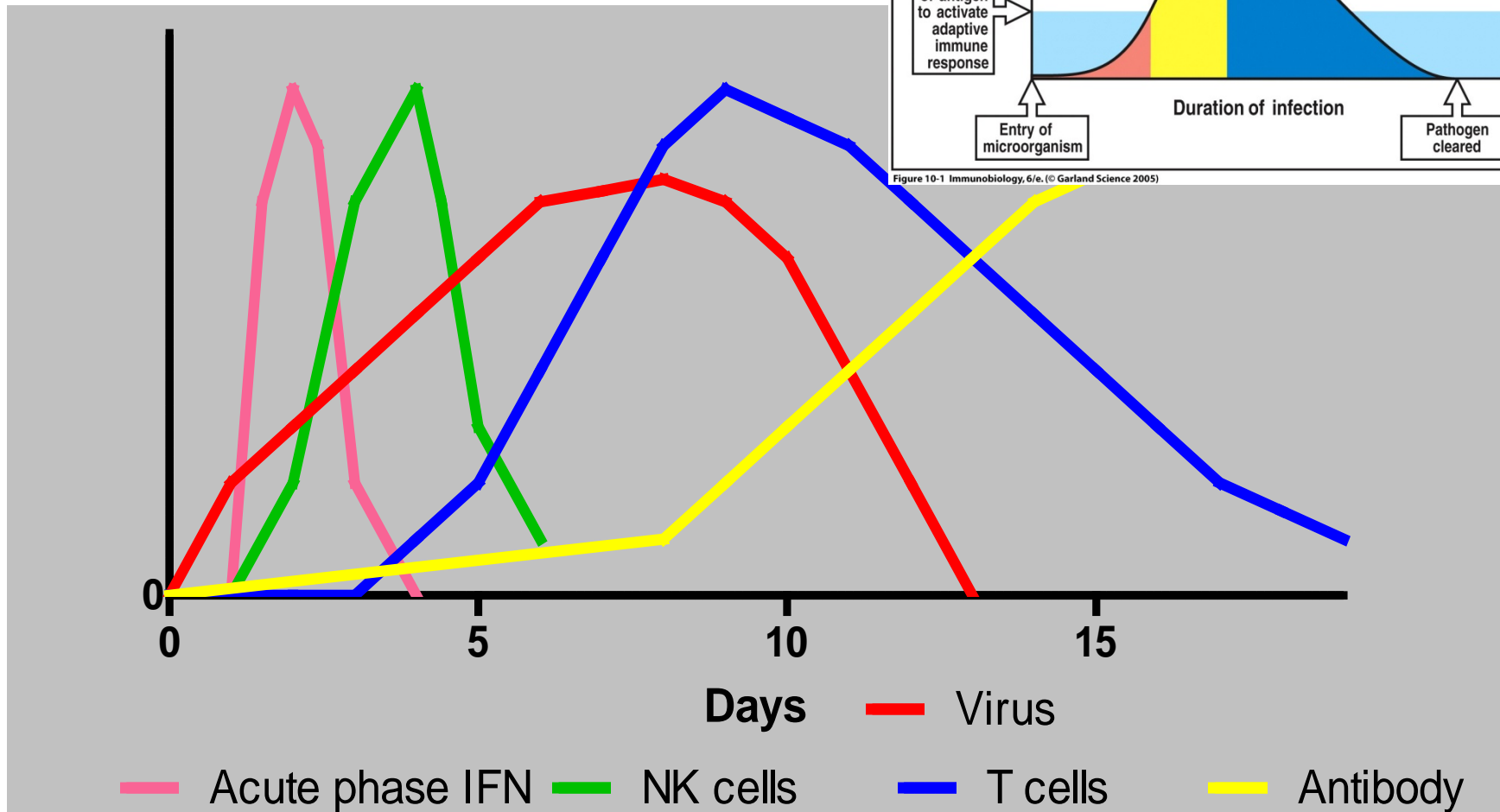


Figure 1-26 Immunobiology, 6/e. © Garland Science 2005

Example of the timing of



The quality of an immune response after vaccination depends on the challenging infection

- Antibodies
 - Toxin
 - Extracellular bacteria
 - Virus
 - Neutralising?
- Cell-mediated immune response
 - Helper (T_H1) T-cells
 - Intracellular bacteria
 - Cytotoxic T-cells
 - Virus (*Lawsonia?*)
 - Often better X-protection between serotypes

Recap of basic vaccinology

Classical / First generation vaccines

- **Live attenuated vaccines (MLV Modified Live Vaccines)**
 - Bacteria
 - Virus
- **Killed whole cell vaccines**
 - Bacteria
 - Virus

Classical vaccines

- **Live attenuated bacteria**
 - Attenuation: Reduced/absent virulence
- Pasteur re-invented vaccines 100 years after Jenner with old cultures of *Pasturella pestis* in chickens
- BCG (bacille Calmette-Guérin)
 - Produced by over 200 repeated subculturings
 - Used since 1929 against tuberculosis
- *Salmonella typhi* (Ty21a)



Classical vaccines

- **Live non-virulent virus**
- **Virus from other species**
 - (e.g. cowpox against smallpox)
- **Naturally occurring attenuated strain**
 - (e.g. Polio type 2)
- **Administration through non-pathogenic route**
- ☹ Virulence genes are often the same that mediate efficient immune activation



Classical vaccines

- **Live attenuated virus**
- **The bonuses:**
 - expresses the full complement of viral antigens
 - induces a broad array of local and systemic immunity
 - provides durable protection
- **The draw-backs:**
 - Chance of reverting to virulent form
 - May be inhibited by maternal antibodies
 - May induce disease and death in immune deficiencies
 - Dangerous production of virulent stock (Ebola, SARS, Foot & Mouth Disease)



Children are vaccinated against measles, mumps and rubella at age 15 months and 4 years with live attenuated virus vaccine

Second generation vaccines

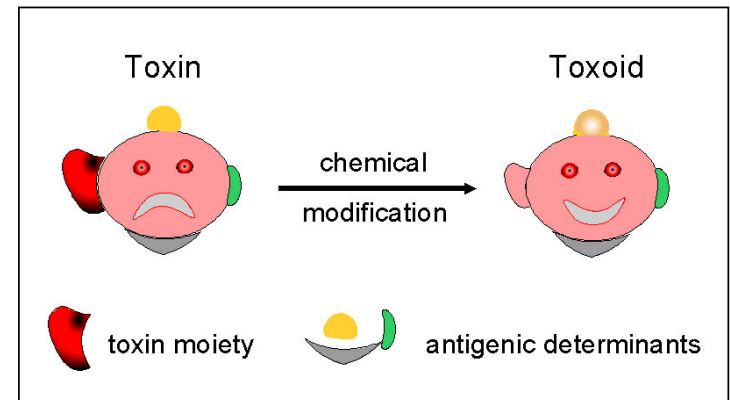
- **Subunit vaccines / Second generation vaccines**
 - Bacterial subunit vaccines
 - Viral subunit and split vaccines
- **Synthetic peptide vaccines**
 - Antibacterial
 - Antiviral
 - Antiprotozoan
 - Against autoimmune diseases

2nd generation subunit vaccines

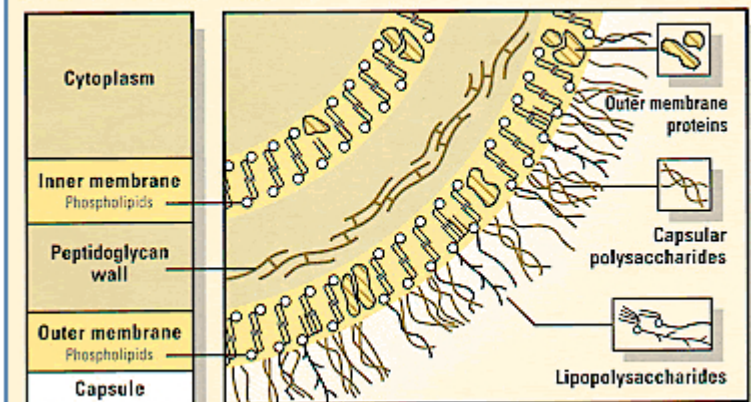
- **Bacterial subunit vaccines**
- **Toxoid vaccines = Inactivated bacterial toxins**
 - Diphtheria, tetanus, pertussis toxoids (whooping cough)
- **Capsular polysaccharide vaccines**
 - *Streptococcus pneumoniae*
 - *Haemophilus influenzae* type b (Hib)

Conjugate vaccines polysaccharide is conjugated to protein to get T cell help

Modification of Toxin to Toxoid



Structure of the cell wall

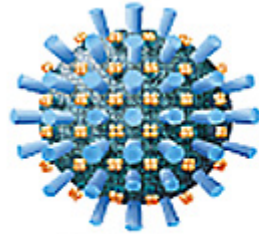


2nd generation subunit vaccines

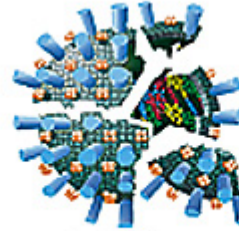
- **Bacterial subunit vaccines**
- **DiTeKiPol/Act-Hib vaccine for all Danish infants**
 - Diphtheria, tetanus, pertussis (whooping cough/kighoste) toxoids
 - Inactivated Polio virus types 1, 2 and 3
 - *Haemophilus influenzae* type b (Hib) polysaccharides conjugated to tetanus toxoid
- **The bonuses:**
 - No chance of original disease
- **The draw-backs:**
 - Requires often adjuvants for efficient immune activation
 - Mostly antibody mediated immunity and no or limited T cell response

2nd generation subunit vaccines

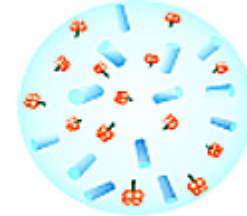
- **Viral subunit and split vaccines**



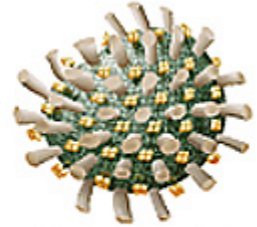
Whole virus



Split virus



Subunit
(surface antigen)



Live attenuated

IFPMA.org

- **Seasonal influenza split vaccine**

- Haemagglutinin (HA) and neuraminidase (NA) antigens isolated from 3 seasonal strains

- **Recombinant produced antigens produced e.g. in yeast**

- **The bonuses:**

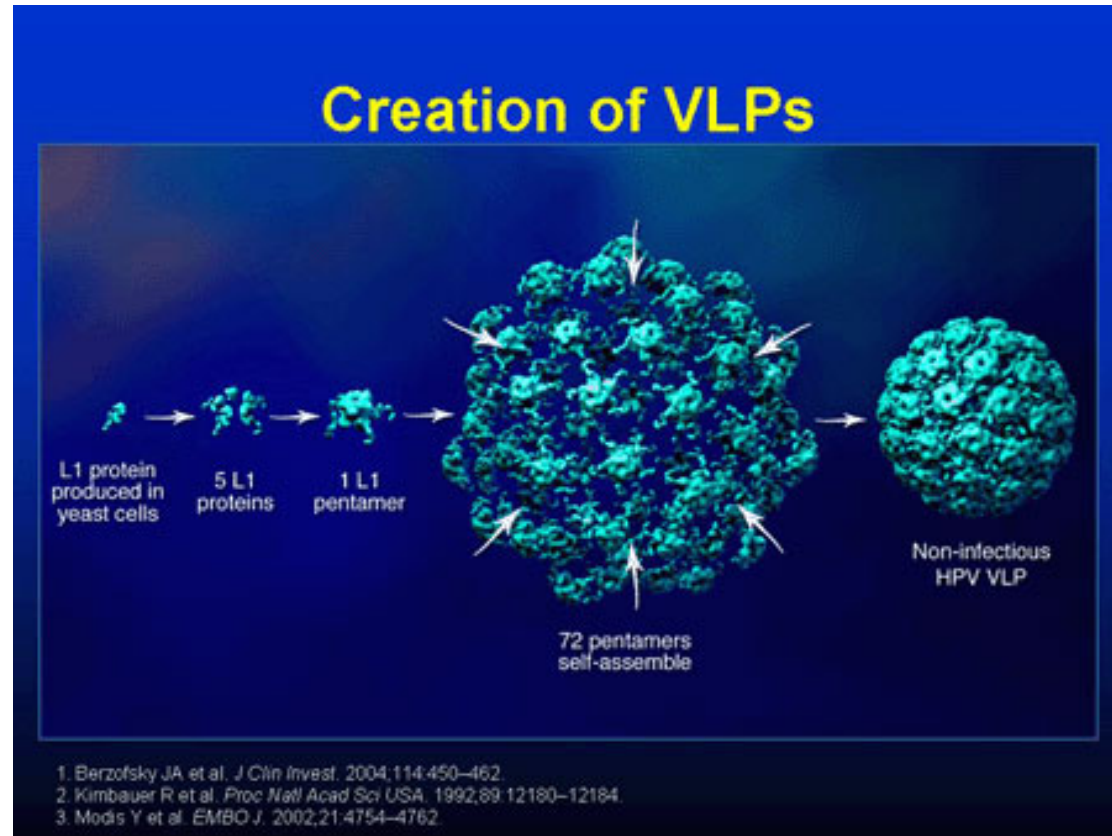
- No chance of original disease
- Fast development of new vaccines for new strains

- **The draw-backs:**

- Requires often adjuvants for efficient immune activation
- Mostly antibody mediated immunity and no or limited T cell response

2nd generation subunit vaccines

- **Virus-like particles (VLPs)**
- Viral structural proteins, such as Envelope or Capsid, can result in the self-assembly of virus like particles
- **Human papilloma virus VLP vaccine**
 - Gardasil (Merck) – in DK
 - Cervarix (GSK)

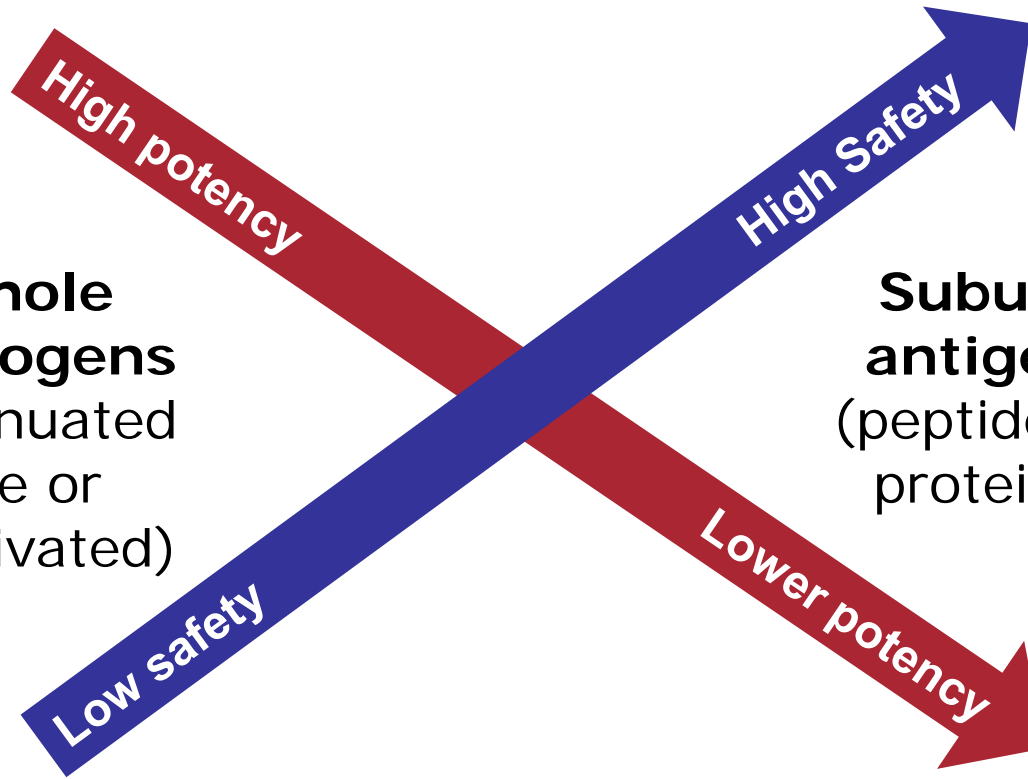
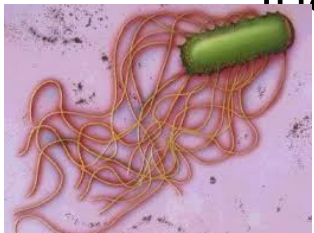


Medscape.org

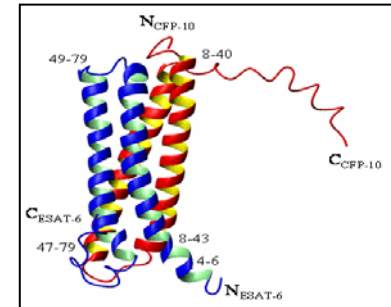
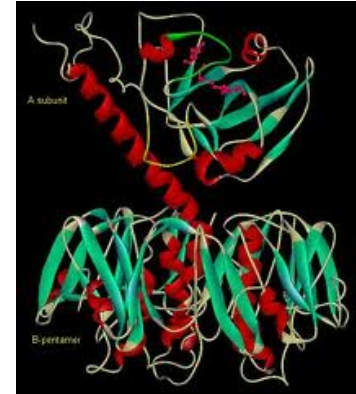
A paradigm shift in Vaccine development



Whole pathogens
(attenuated live or inactivated)



Subunit antigens
(peptides or proteins)



Adjuvants are needed in subunit vaccines to improve immunogenicity.

Vaccine adjuvants

Adjuvant: substances or compositions enhancing the quantity and quality of immune response to another substance

Immunomodulator: a compound having a separate, longer lasting effect on the immune system

Adjuvants may be immunomodulators or contain additional immunomodulators, e.g. AS04: Alum + MPL (TLR4 agonist)

Adjuvants alone are not licensed; each specific antigen/adjuvant formulation is licensed as a product

Third generation vaccines

- **Recombinant vaccines**
 - Live bacterial vector
 - Live viral vector
 - Synthetic recombinant vaccines

- **DNA/RNA vaccines**

Recombinant vaccines

- **Live bacterial vectors**

- Deletion of virulence genes and incorporation of foreign genes to express whole proteins.
- ☹ Virulence genes are often the same that mediate efficient immune activation
- ☹ Difficult to express correctly folded virus surface proteins

- *Vibrio cholerae*
- *Salmonella / Shigella*
 - Easily incorporates plasmids – may be transferred to other bacteria?
 - Oral vaccination

Recombinant vaccines

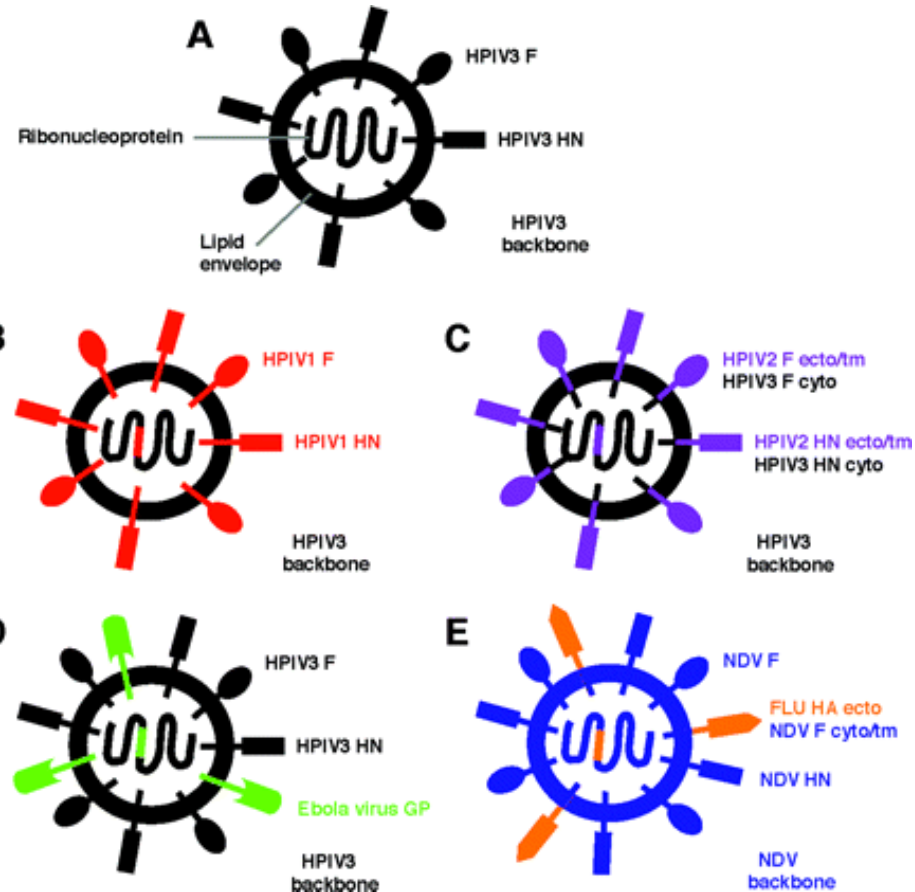
- **Live viral vectors**

- **Antigenic chimeric viruses**

- Replace surface antigen(s) of the vector with that of the pathogenic virus

- **Vectored virus vaccines**

- foreign genes inserted in the genome to express large quantities of protein by infected cells



Human parainfluenzavirus type 3 (A) as antigenic chimera virus (B and C) expressing glycoproteins from other serotypes, or as vectors expressing added genes from other viruses (D and E)
Bukreyev et al. Journal of Virology, November 2006

Recombinant vaccines

- **Live viral and bacterial vectors**

- **Poxvirus vector**

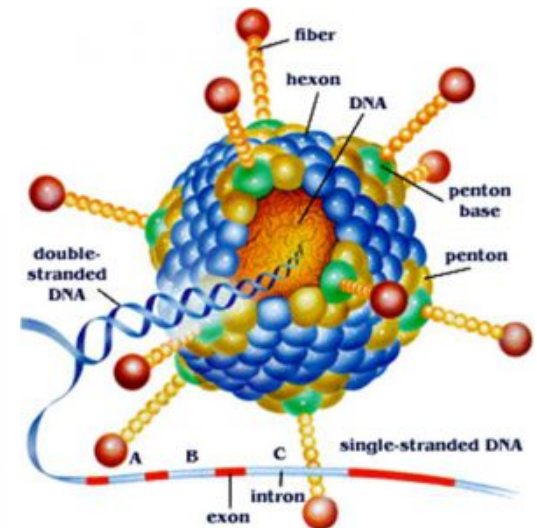
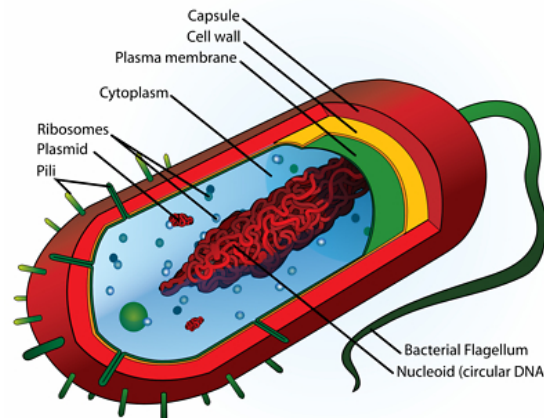
- Smallpox and modified vaccinia ankara (MVA) virus vaccines
- Can host large foreign genes in the genome

- **Adenovirus vector**

- Many strains available
- Mucosal delivery

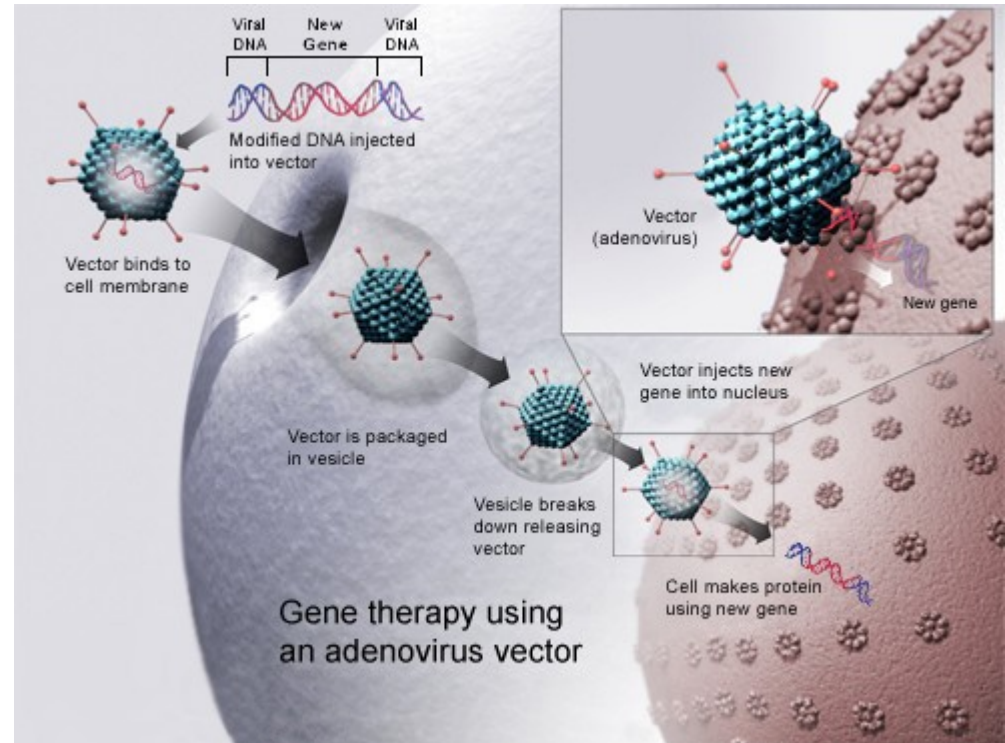
- **E. Coli or Salmonella**

- Mucosal delivery



Plasmid DNA vaccines

- A "by-product" of failed gene therapy
- Selected gene from pathogen is transfected into host cells
- All cell products are presented as peptide fragments in MHC I on cell-surface
- Secreted products are phagocytised and presented in MHC II
- Immune cells recognise the foreign antigen and generates an immune response



Immunological correlates of protection

- Can we use laboratory tests to measure vaccine efficacy?

Standard measurement of antibody response

Antibody level

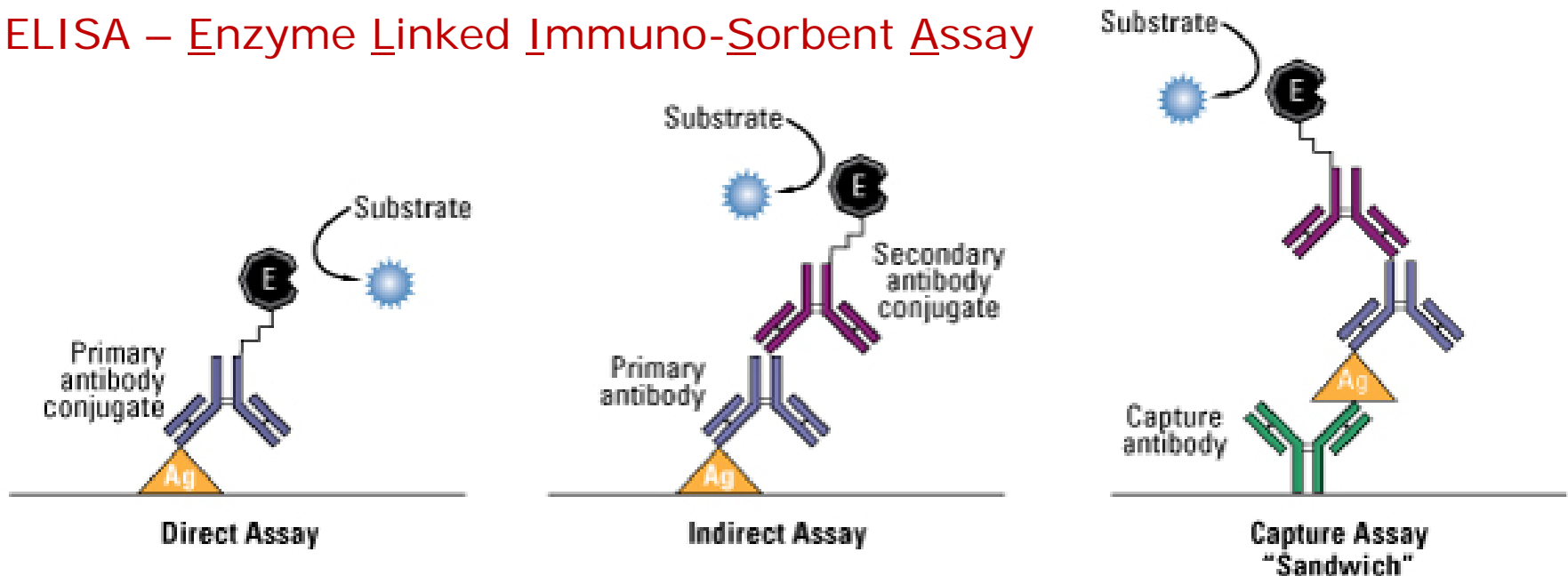
Maybe a particular antibody isotype

Mucosal sIgA response (hard)

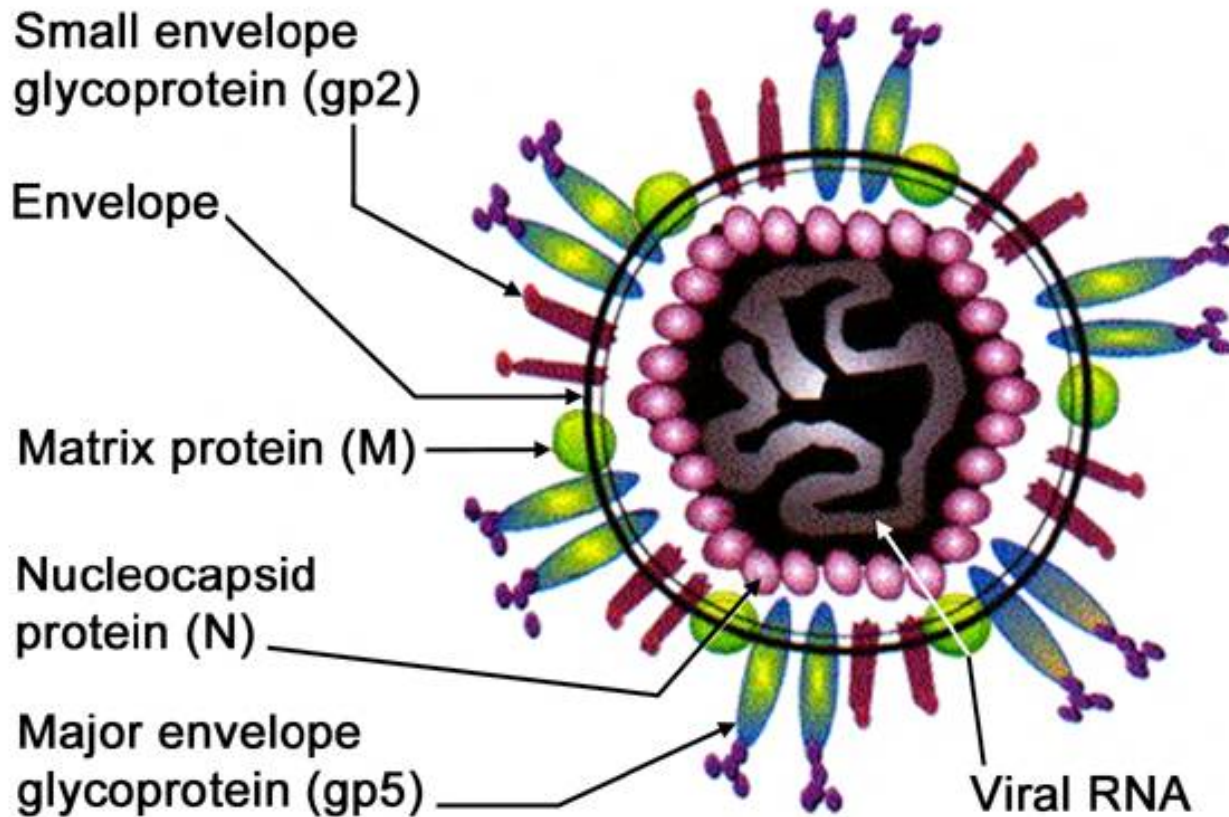
Neutralising titer

E.g. Inhibition of infection in cell culture

ELISA – Enzyme Linked Immuno-Sorbent Assay



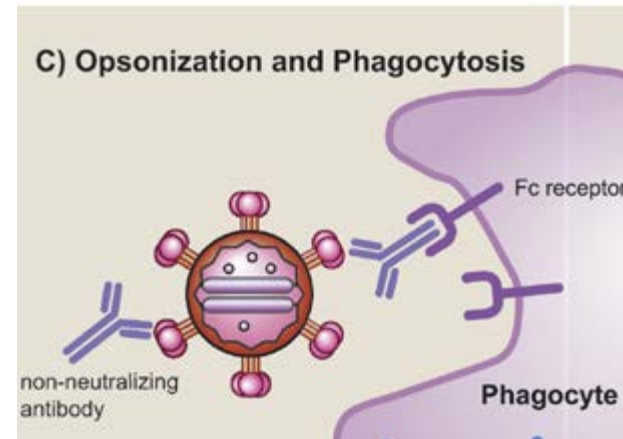
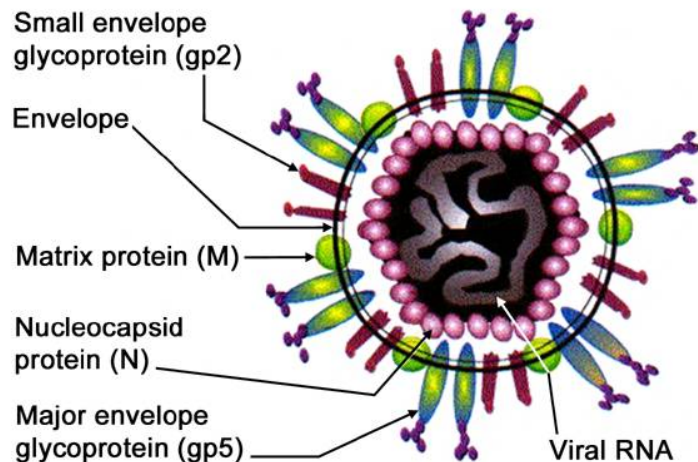
Example: PRRS



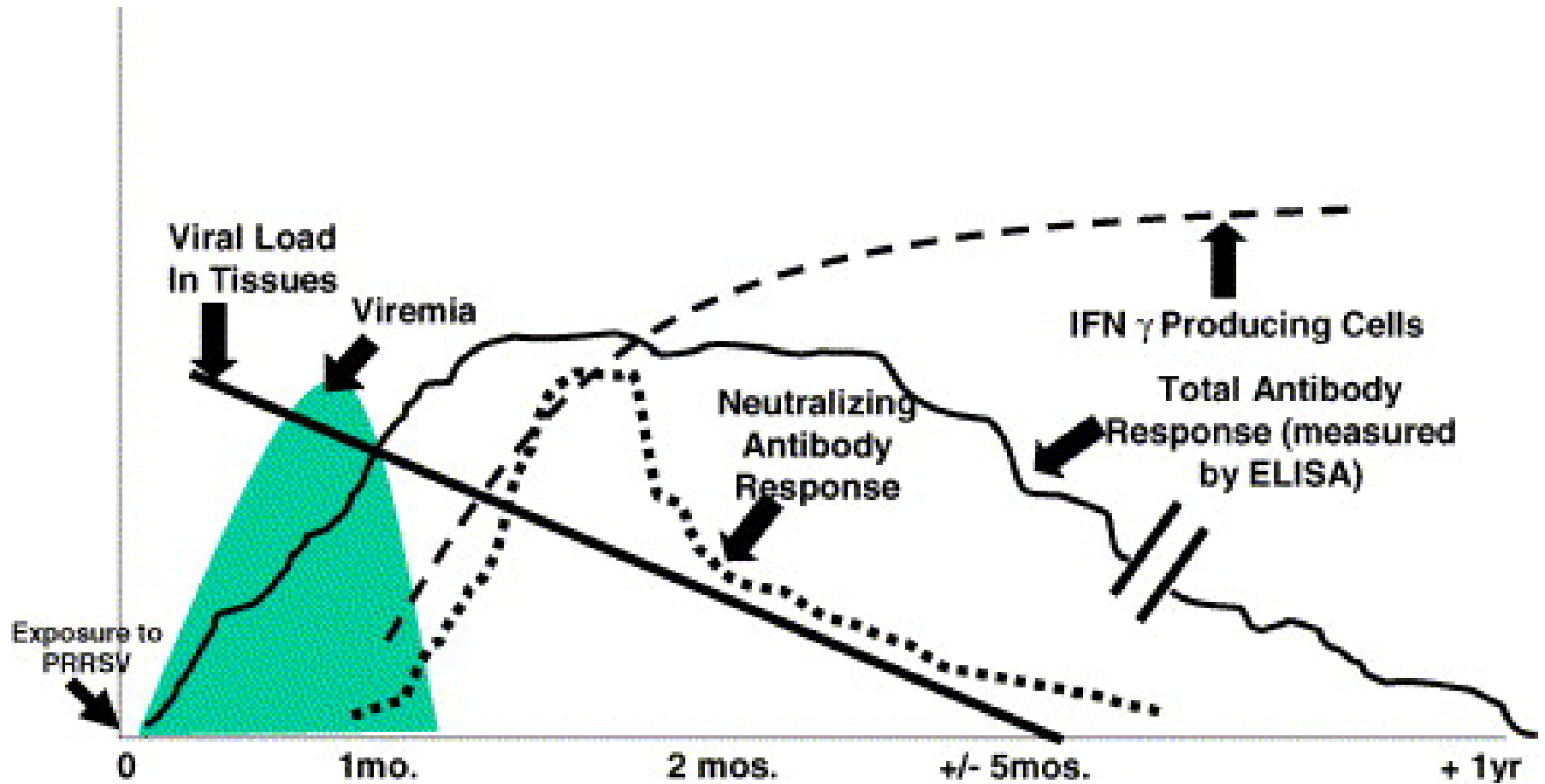
PRRS Antibodies:

The good, the bad and the ugly

- **The Good:** Neutralising antibodies against GP5 protein
- **The Bad:** Irrelevant antibodies against nsp2, N and M protein
- **The Ugly:** Antibodies (N and GP5) increasing the uptake of infective virus in macrophages (ADE)



ADE: Antibody Dependent Enhancement



Development of immunological event after PRRSV infection

Lopez OJ, Osorio FA. Role of neutralizing antibodies in PRRSV protective immunity. *Vet Immunol Immunopathol.* 2004 Dec 8; 102(3): 155-63.

Measures of T_H1 immune responses

Intradermal skin test *In vivo* assay

DTH – delayed type hypersensitivity reaction

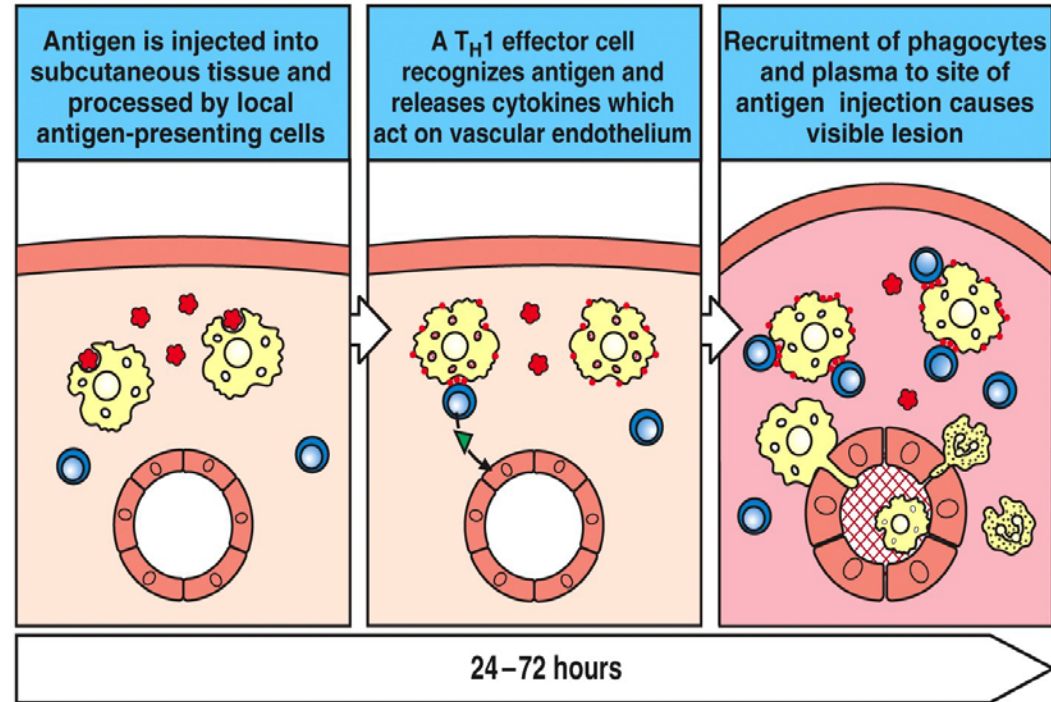


Figure 12-25 Immunobiology, 6/e. (© Garland Science 2005)

Measures of T_H1 immune responses

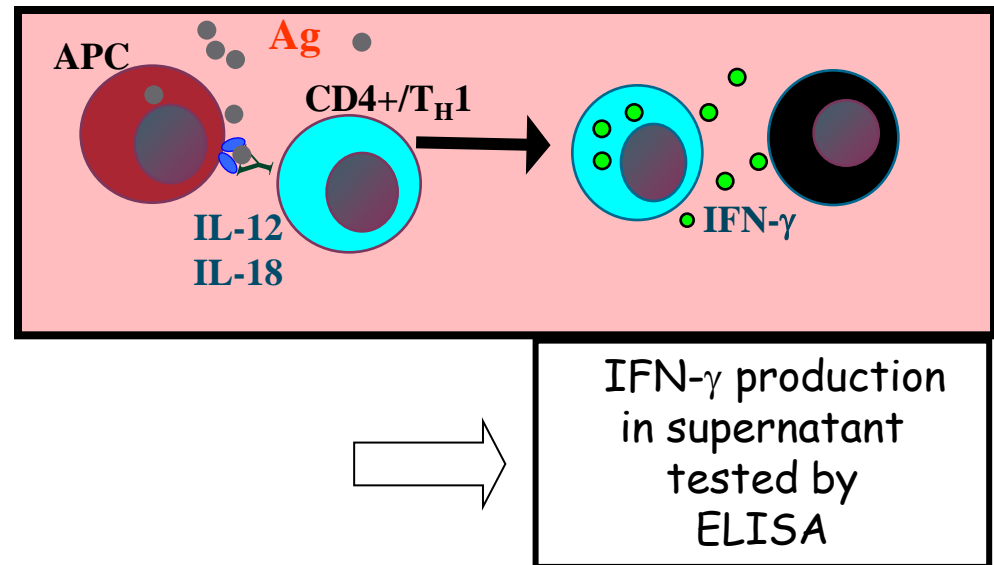
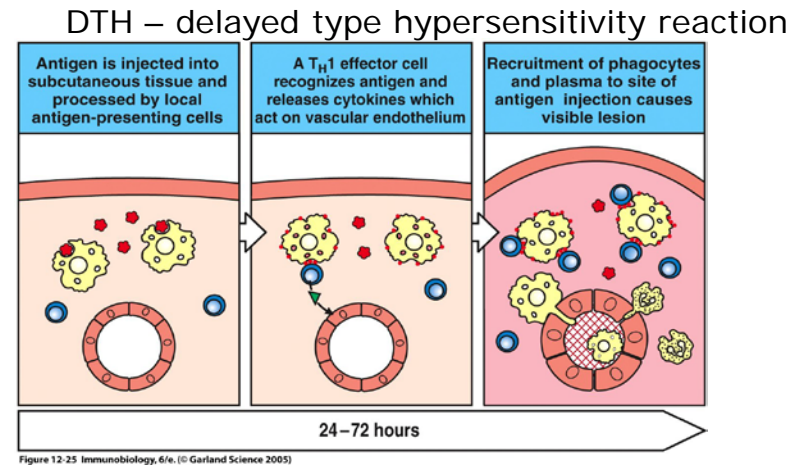
Intradermal skin test

In vivo assay

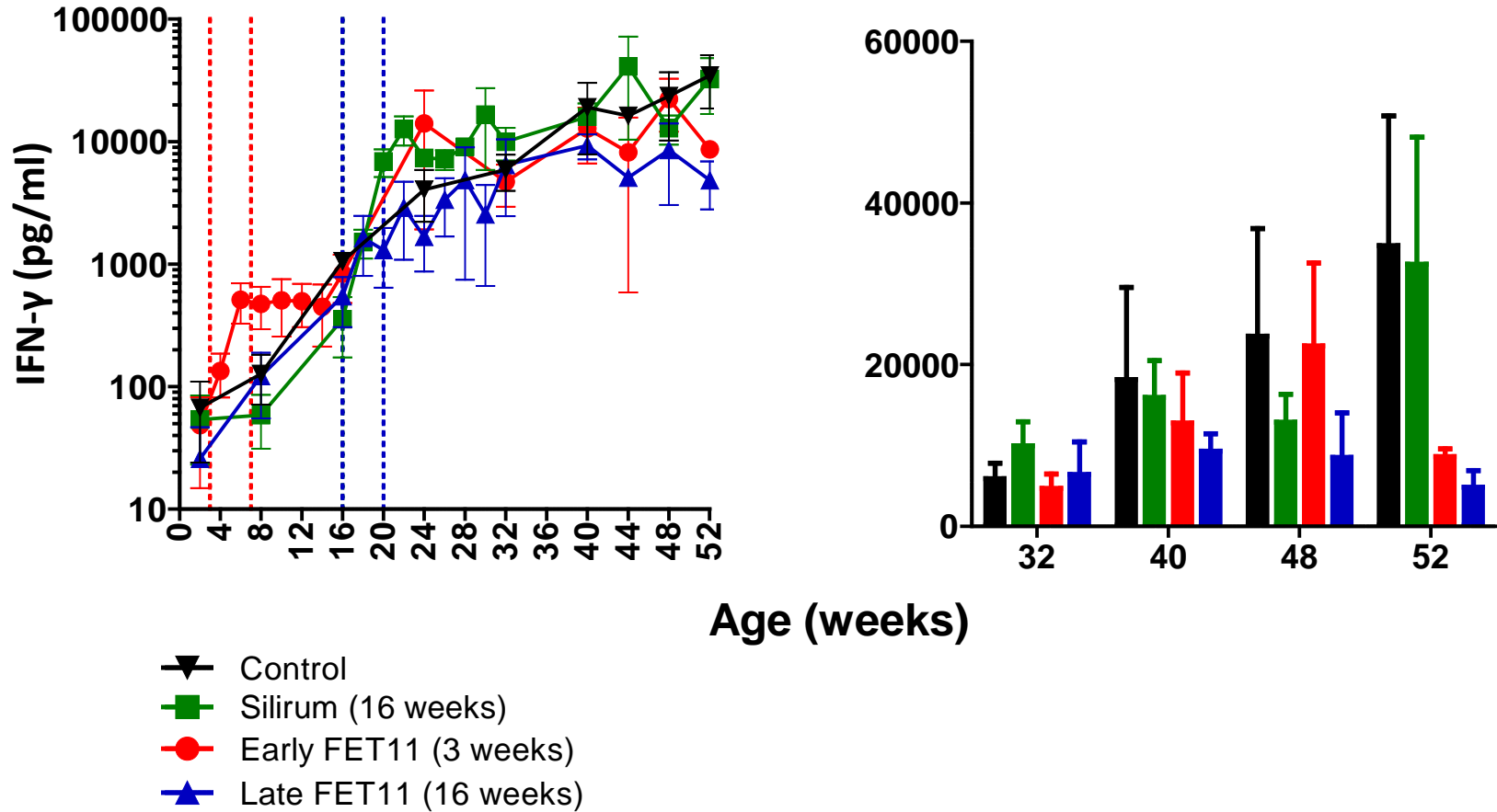
Interferon-gamma (IFN- γ) release assay

PBMC or heparin stabilised whole-blood cultures are incubated for 20-22 hours with antigens. Any T_H1 cells recognising the Ag will produce → **IFN- γ** (ELISA)

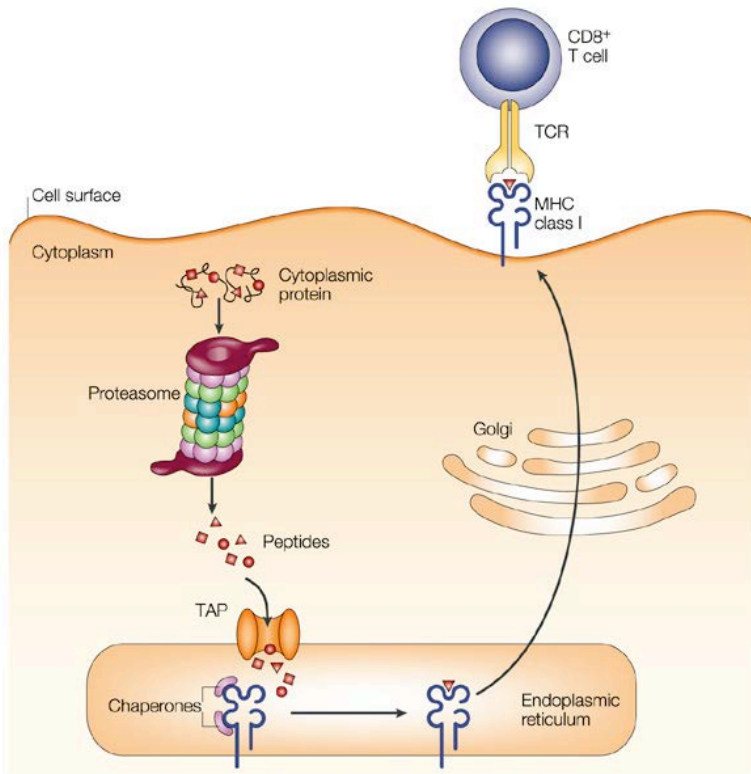
In vitro assay



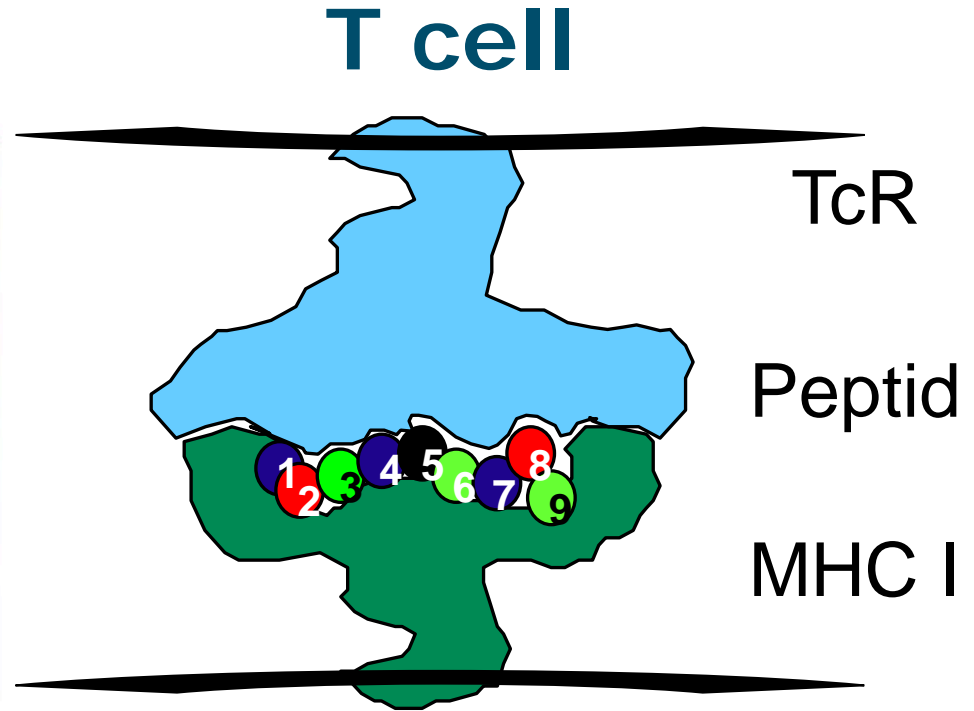
Paratuberculosis-specific IFN- γ = a Surrogate of Infection



Measuring cytotoxic T cells (CTL)

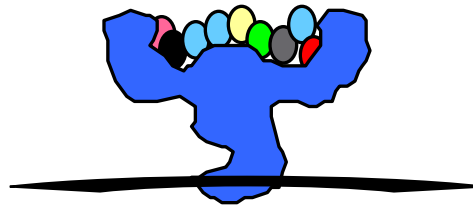
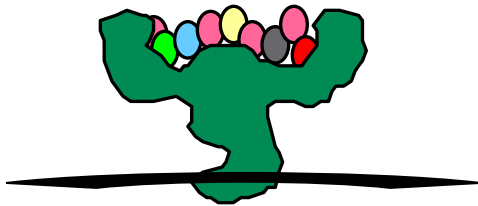
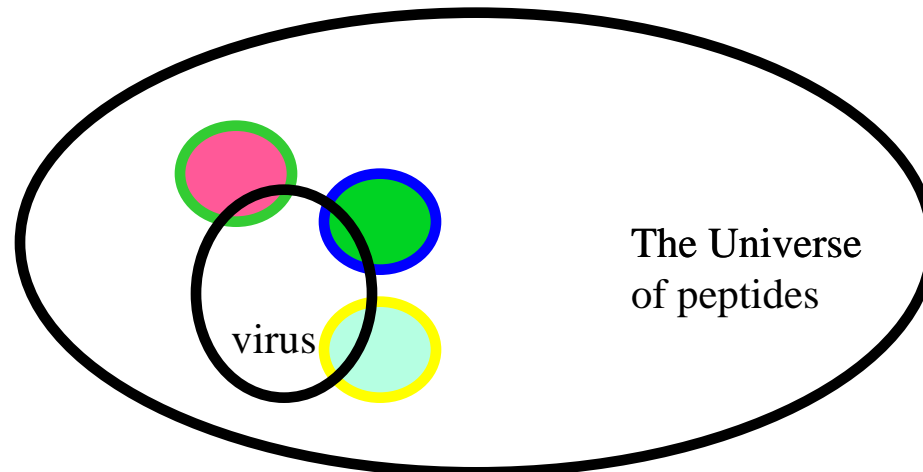


Nature Reviews | Immunology



Infected cell

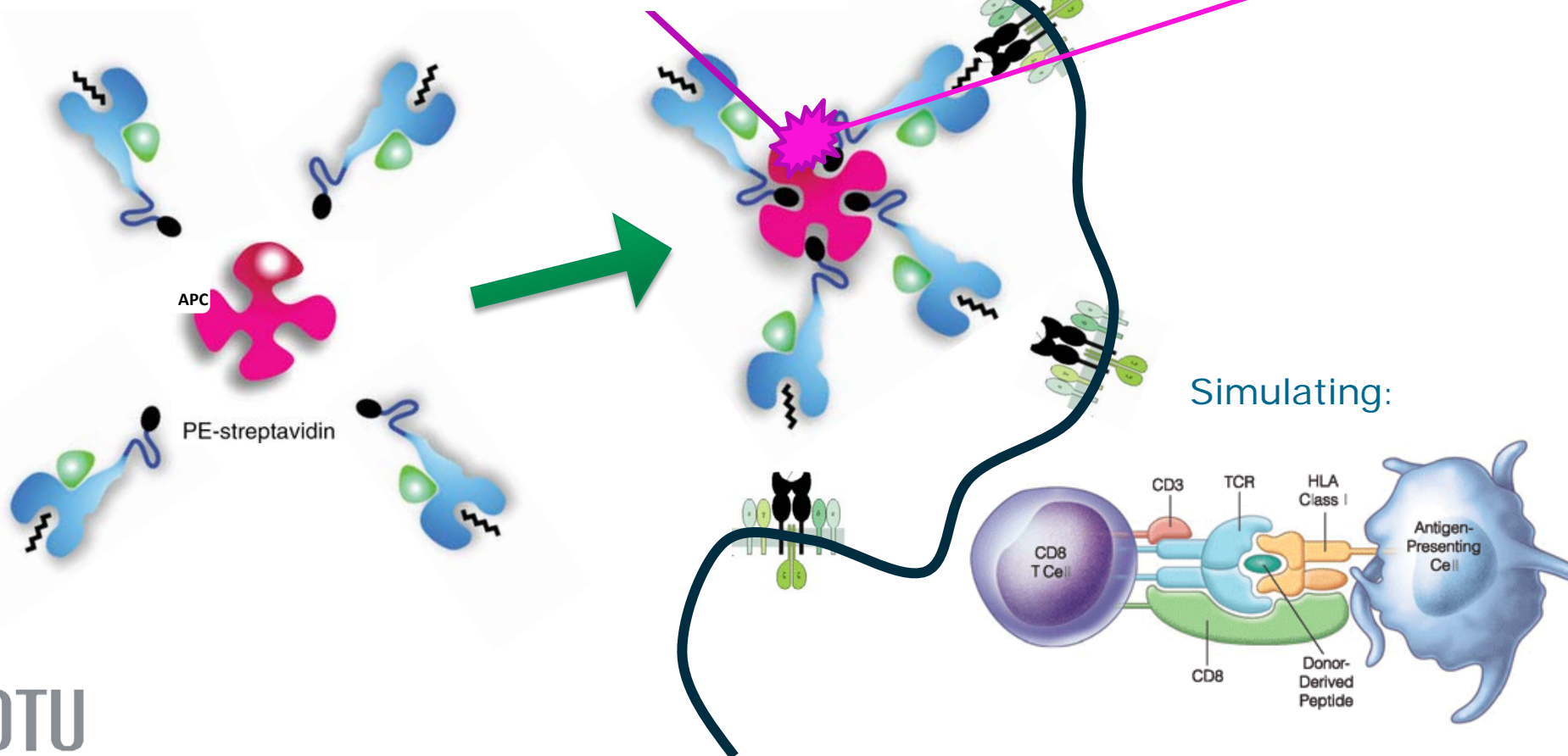
MHC polymorphism and immune specificity



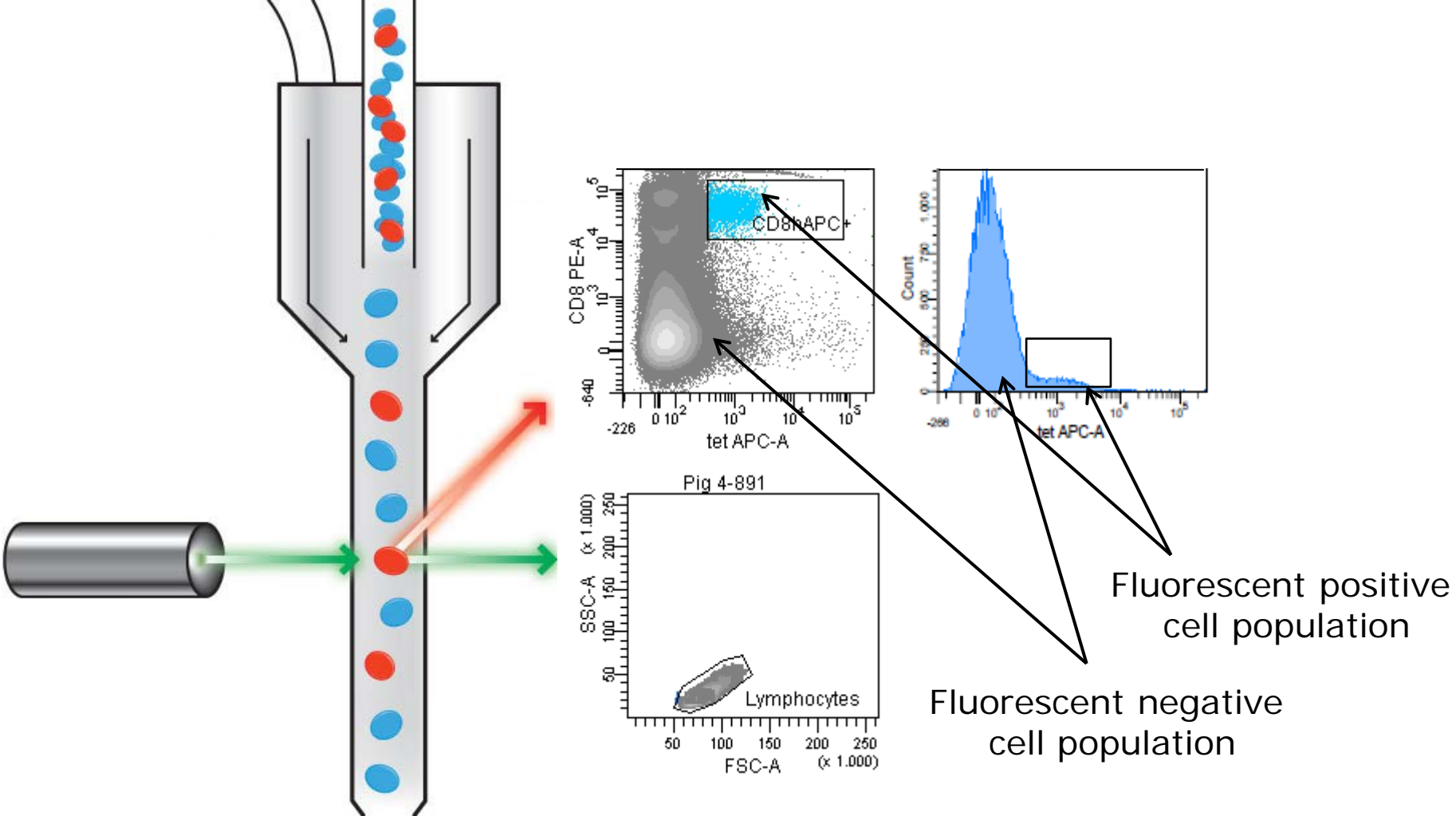
SLA tetramers:

Ex vivo identification of T cell epitopes

- Recombinant produced MHC class I molecules complexed with virus peptide
- Assembled in tetra- or multimer structures
- Direct staining of Ag-specific CTLs



Flow cytometry (FACS) measurement of labelled cells



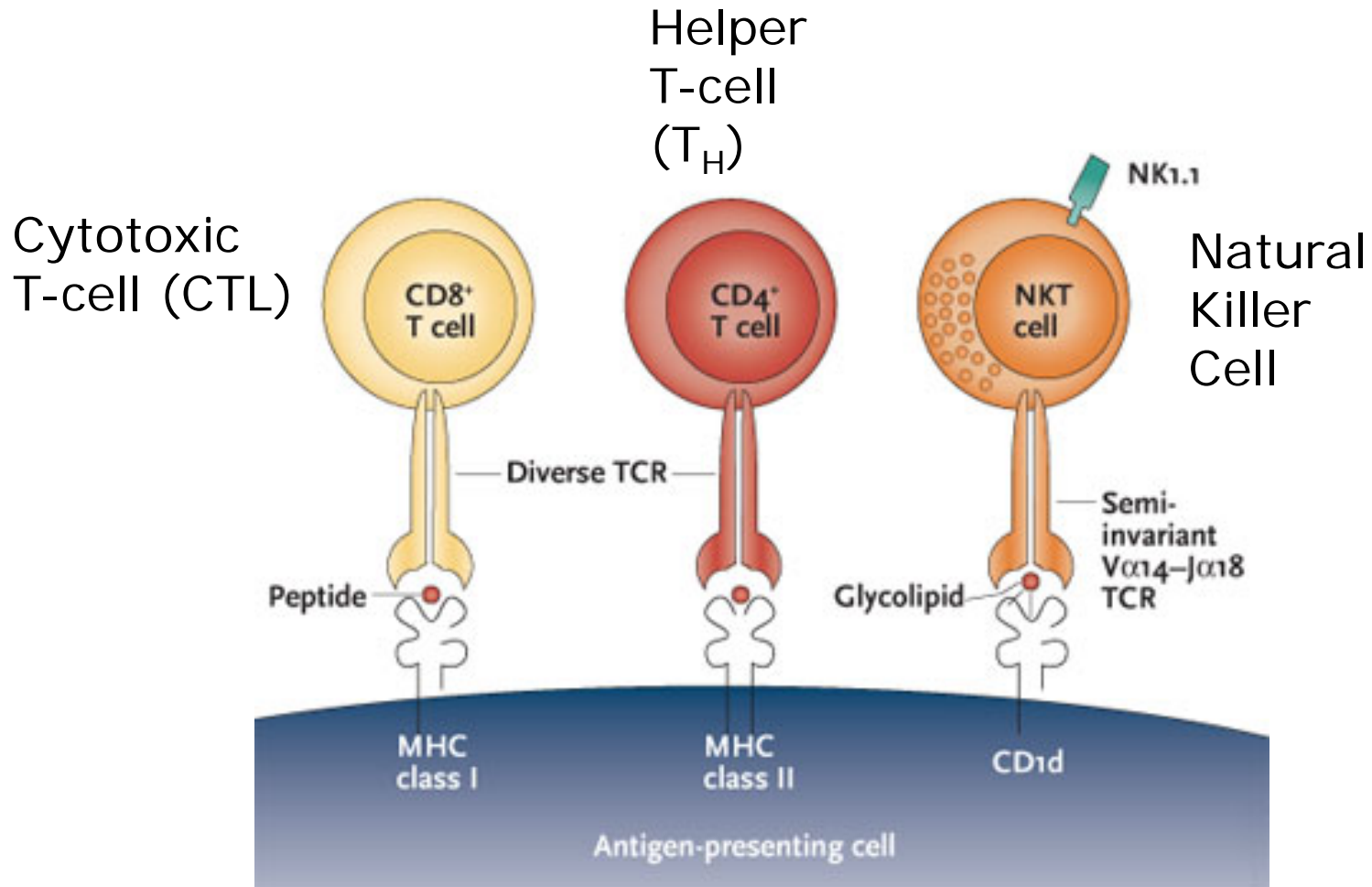
What do you know about vaccinations for you and your children?

- GROUP WORK:
- Sit 3-4 together and fill out as many **details** about the **vaccines you have been given**.
- Use your experience and the internet:
 - www.ssi.dk
 - Wikipedia
 - etc
- **Start again at 10.35**

What's new in the field of Veterinary Vaccines

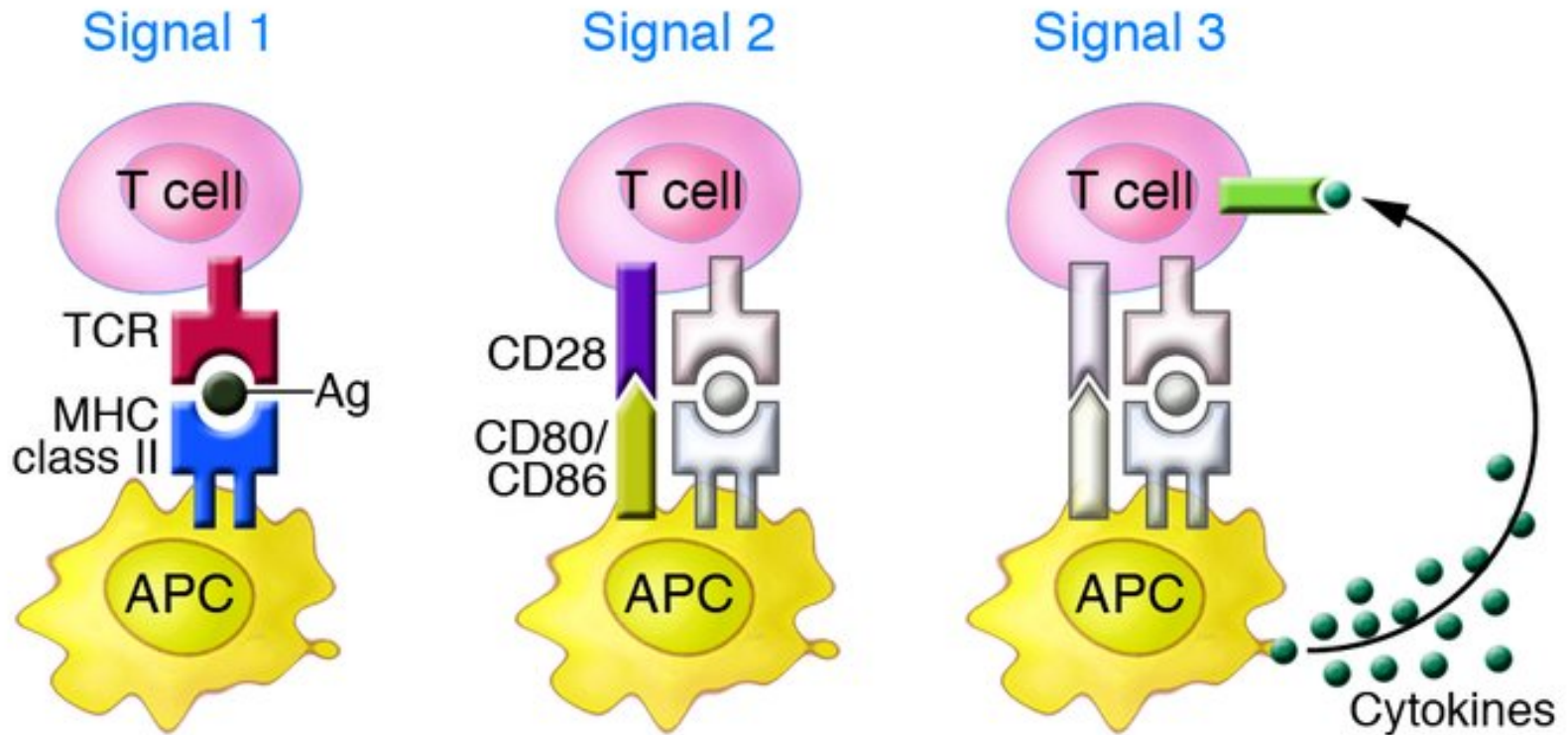
- Developments in Adjuvants

Three effector T-cells



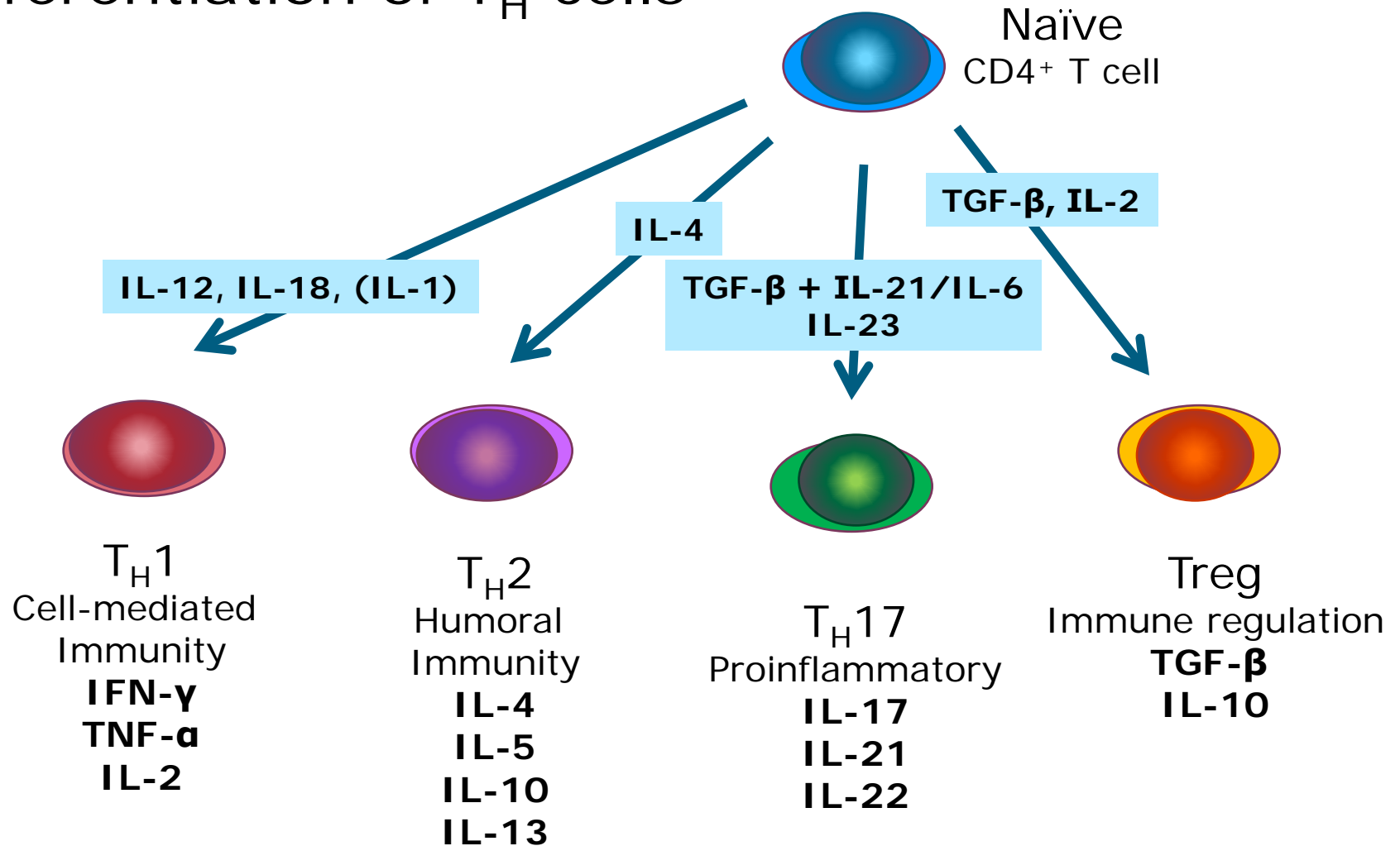
www.the-scientist.com

Three-signal activation of T-cells



Gutcher & Becher *J Clin Invest.* 2007

Cytokines drive the differentiation of T_H cells



4 signals for immune activation = 4 targets for adjuvants

Signal 1: The Antigen presentation

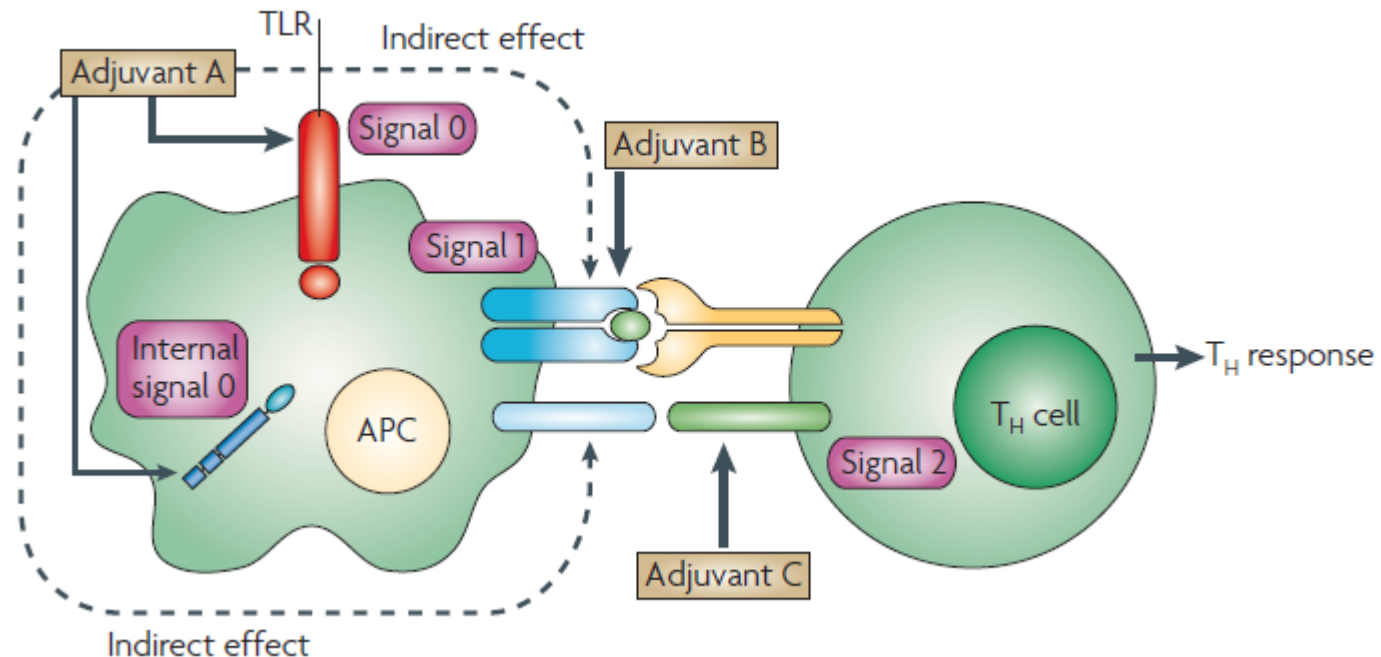
- delivery of Ag to APC or MHC class I cross-presentation

Signal 2: Receptor-ligand interaction between APC and T cell

- target CD40, CD80, CD86 upregulation

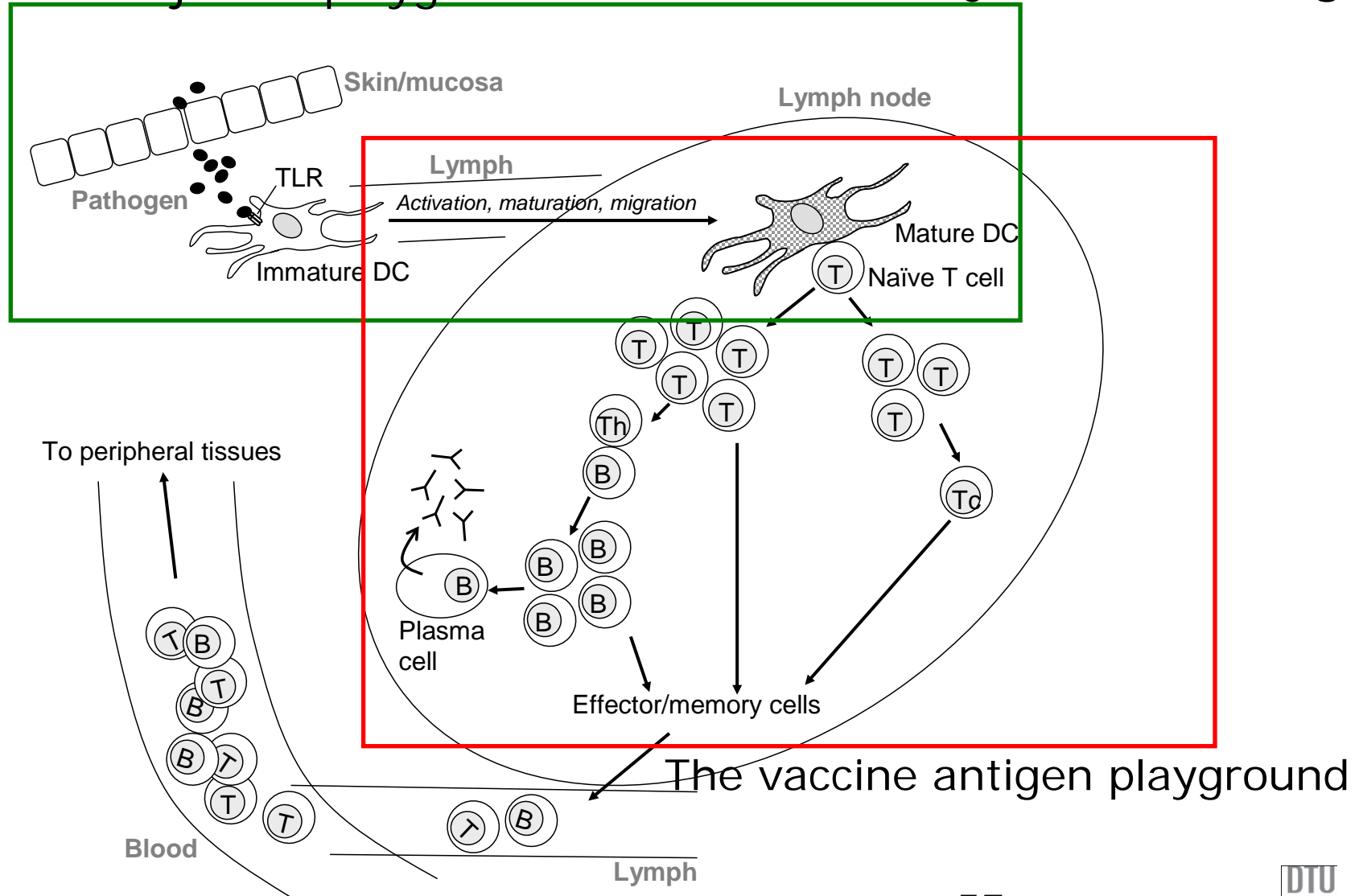
Signal 0: Activation of the APC Signal 3: cytokine co-stimulation of the T cell

- e.g. TLR agonists, co-delivery of cytokines and immunomodulatory molecules



Guy, Nat Rev Microbiol 2007

The adjuvant playground VACCINES: adjuvant + antigen



Emperically-based adjuvants: crude and rude but they work (we just don't really know how..)

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

NB: Water-in-oil plus emulsifier!



+MF59

Figure A-4 Immunobiology, 6/e. (© Garland Science 2005)

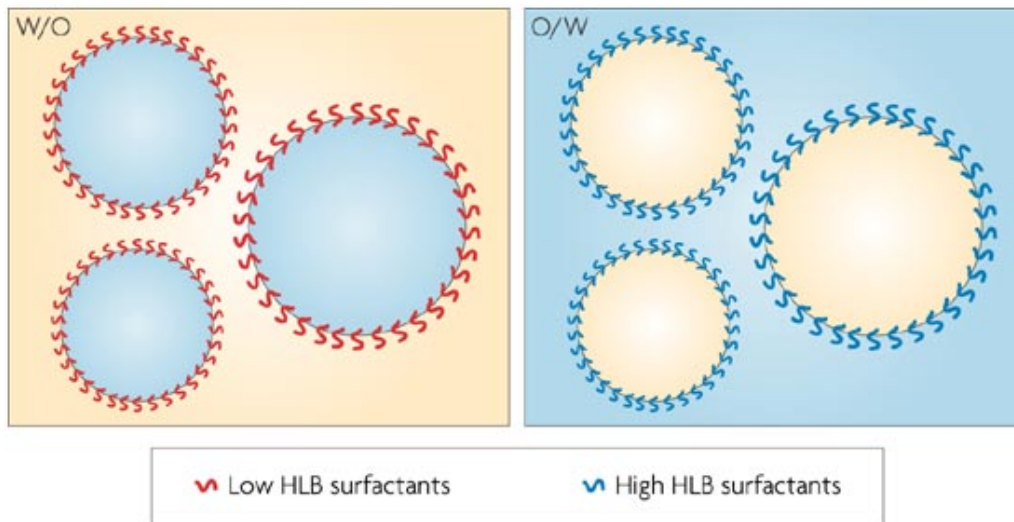
Time matters – Depot effect

Antigen depot with promoted persistence, stability, conformational integrity and slow release increase immunogenicity.

Controlled release may eliminate need for booster vaccine.

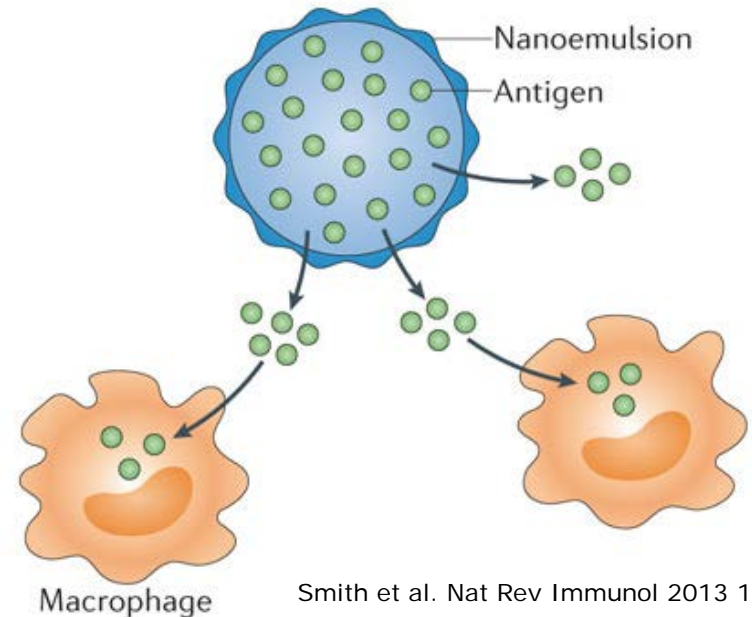
For many years (wrongly) believed to be main Alum mechanism of action

Water-in-oil or Oil-in-water formulations



Guy, Nat Rev Microbiol 2007

Nanoparticle droplets



Smith et al. Nat Rev Immunol 2013 13:701



Alum: Three proposed mechanisms

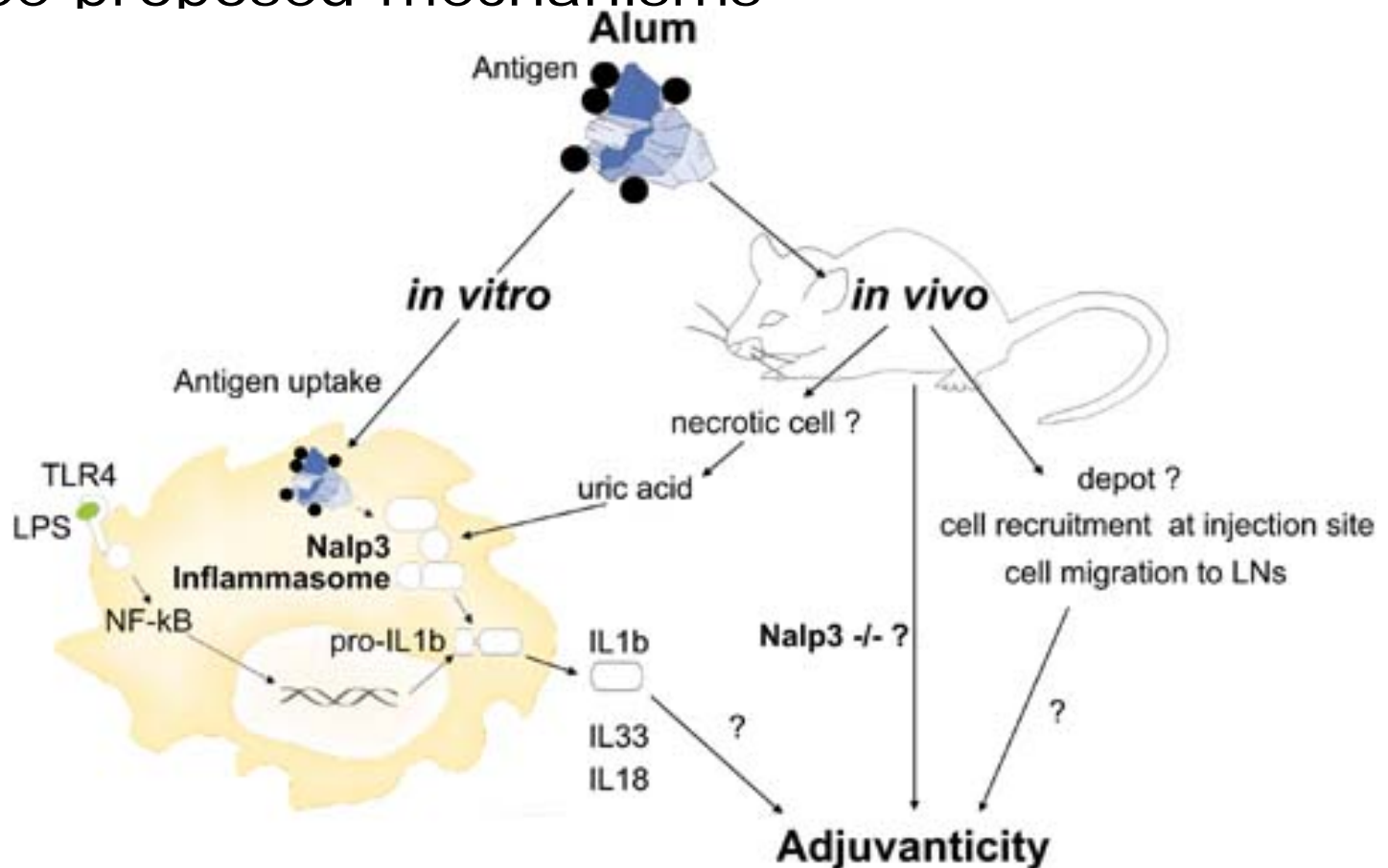
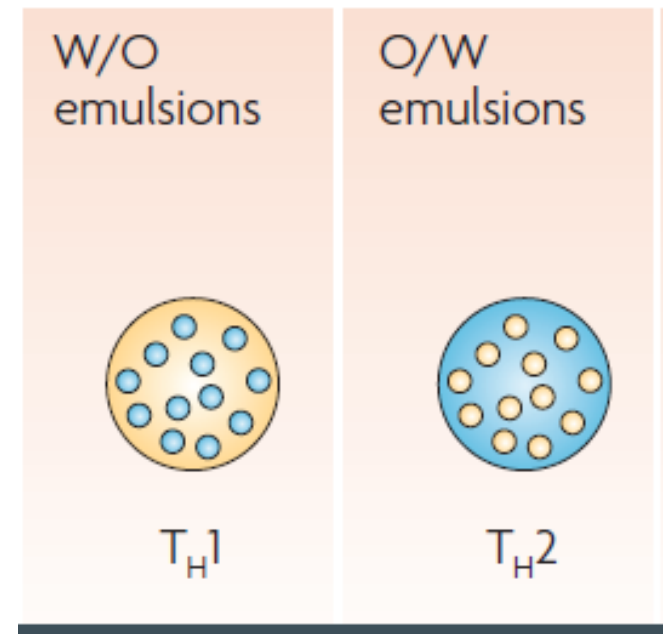


Figure 2. Proposed mechanisms of action of alum *in vitro* and *in vivo* and their possible contributions to adjuvanticity. *In vitro*, alum complexed with antigen increases antigen uptake by APC. In addition, alum induces direct activation of Nlrp3 (Nalp3) inflammasome complex and synergizes with LPS stimulation of TLR4 for the secretion pro-inflammatory cytokines such as IL-1 β , IL-18 and IL-33. *In vivo*, alum induces necrosis in unidentified target cells resulting in production of uric acid, which has the potential to stimulate Nlrp3. Alum also stimulates local recruitment of APC and migration of APC to the draining lymph nodes. It has been proposed that alum may also enhance local antigen persistency ("depot" effect). The contribution of all these activities to alum adjuvanticity and the requirement of Nlrp3 are not yet fully understood.

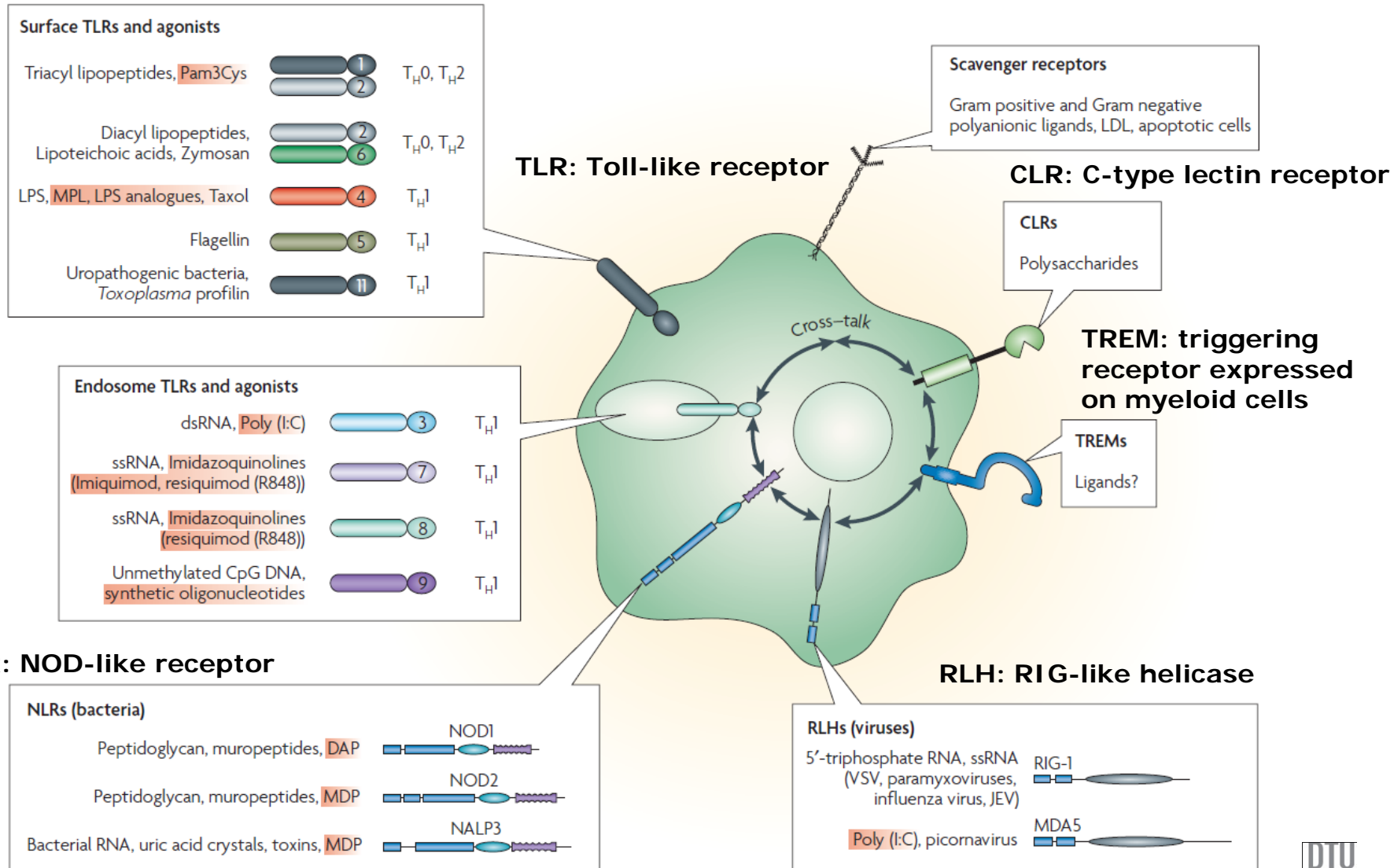
Emulsion adjuvants

- **Emulsion** (dispersion of two non-miscible fluid phases with each other), water phase droplets in oil phase, or opposite, stabilized by surfactant
- **Surfactant:** hydrophilic/lipophilic balance (HLB); low HLB (hydrophobic) has tendency to solubilize in oil phase – creates WATER-in-OIL emulsions high HLB (hydrophilic) has tendency to solubilize in water phase – creates OIL-in-WATER emulsions

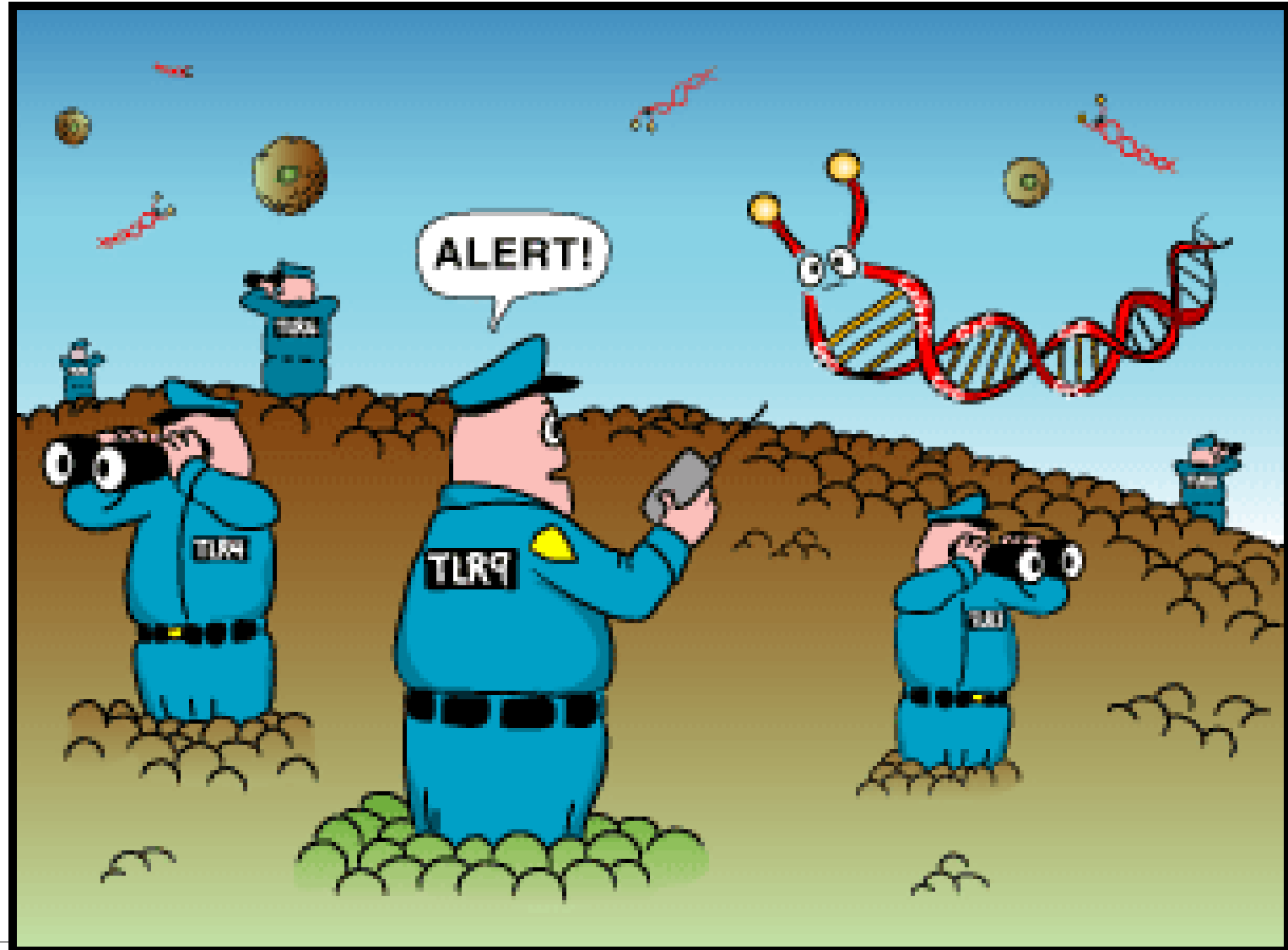


Danger (inflammation) matters

Danger sensing molecules are scattered on Antigen-presenting cells (APCs)



DNA-attack: Innate sensors recognize danger...



Size matters

Aggregate/particle size (mean diameter)

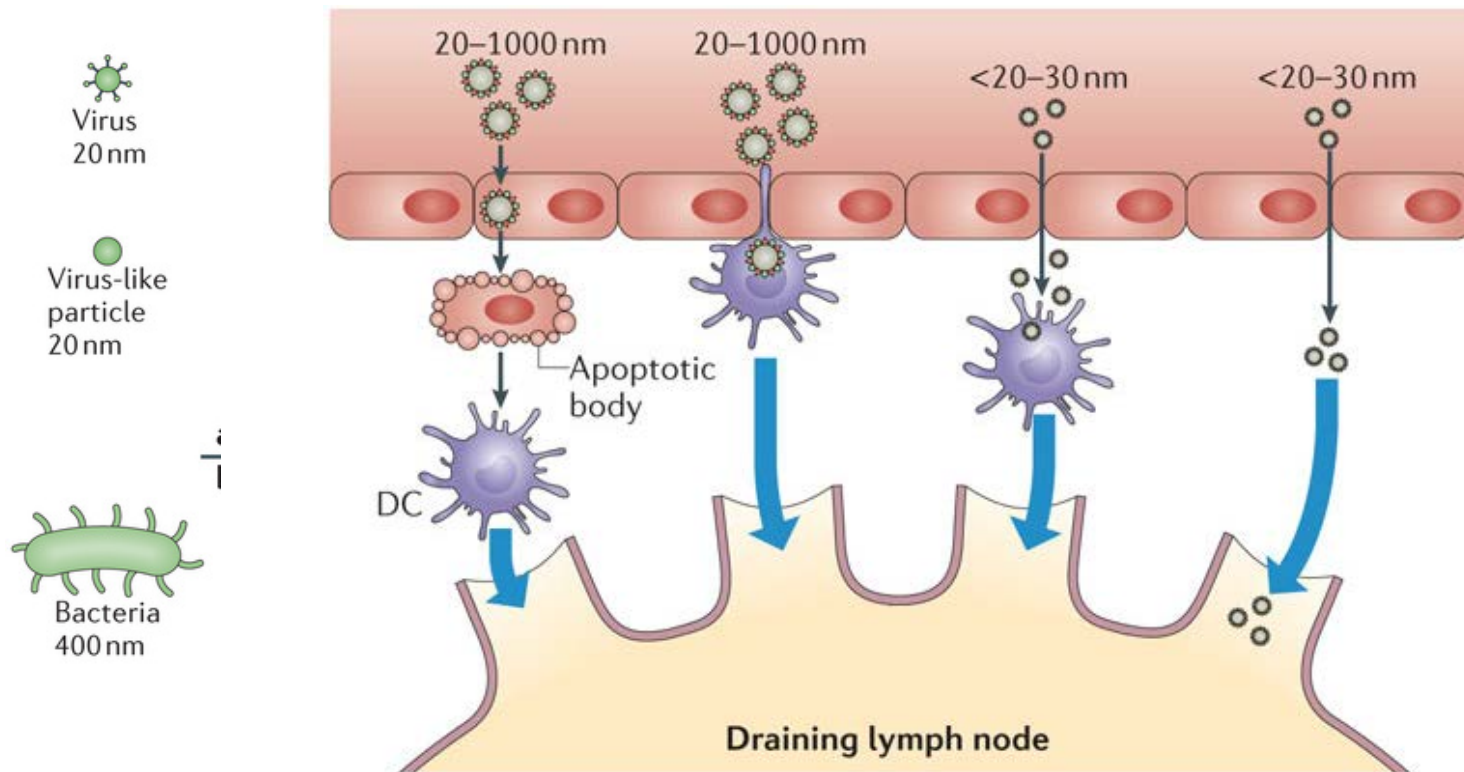
- > 10 μm
- 200nm -10 μm
- 40-80 nm
- < 25 nm

Proposed mechanism

- Antigen depot, slow release
- APC uptake and migration to LN
- Facilitated DC uptake
- Direct trafficking to LN

Result

- Ab
- IL-4/ T_H2
- IFN- γ / T_H1
- CMI(CD8?)

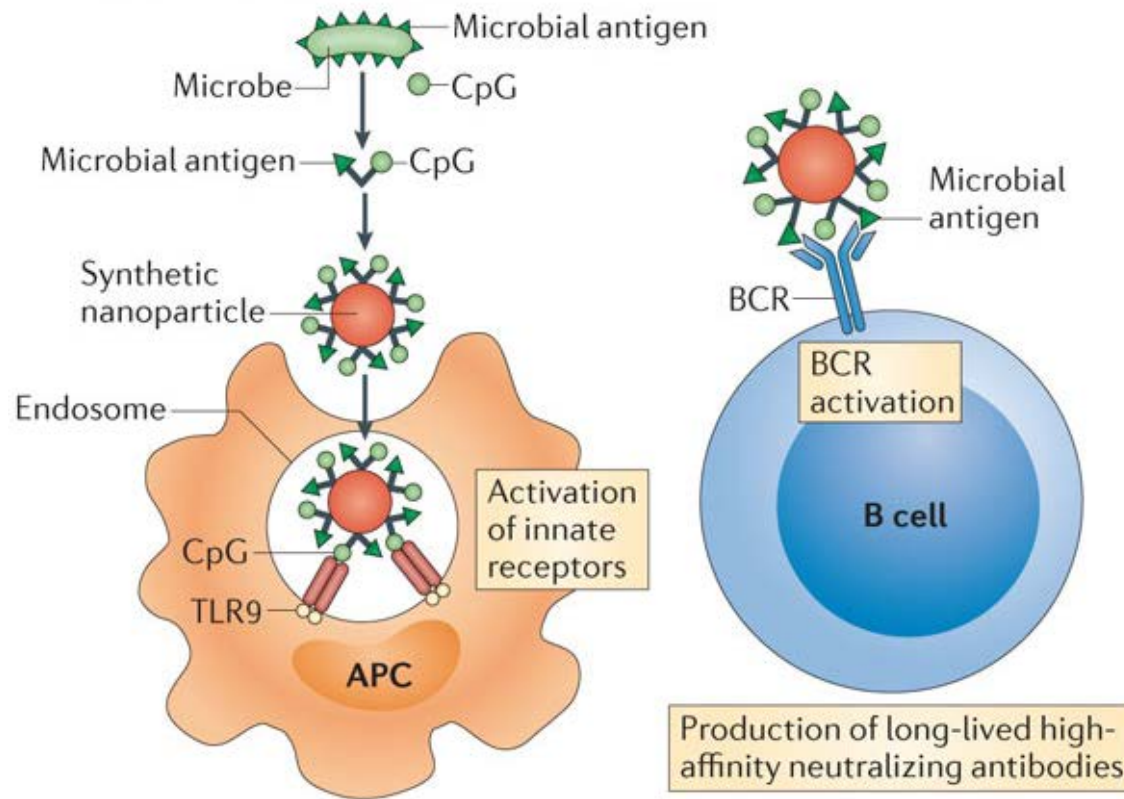


Repetition matters

Repeated display of epitopes or TLR ligands increase immunogenicity.

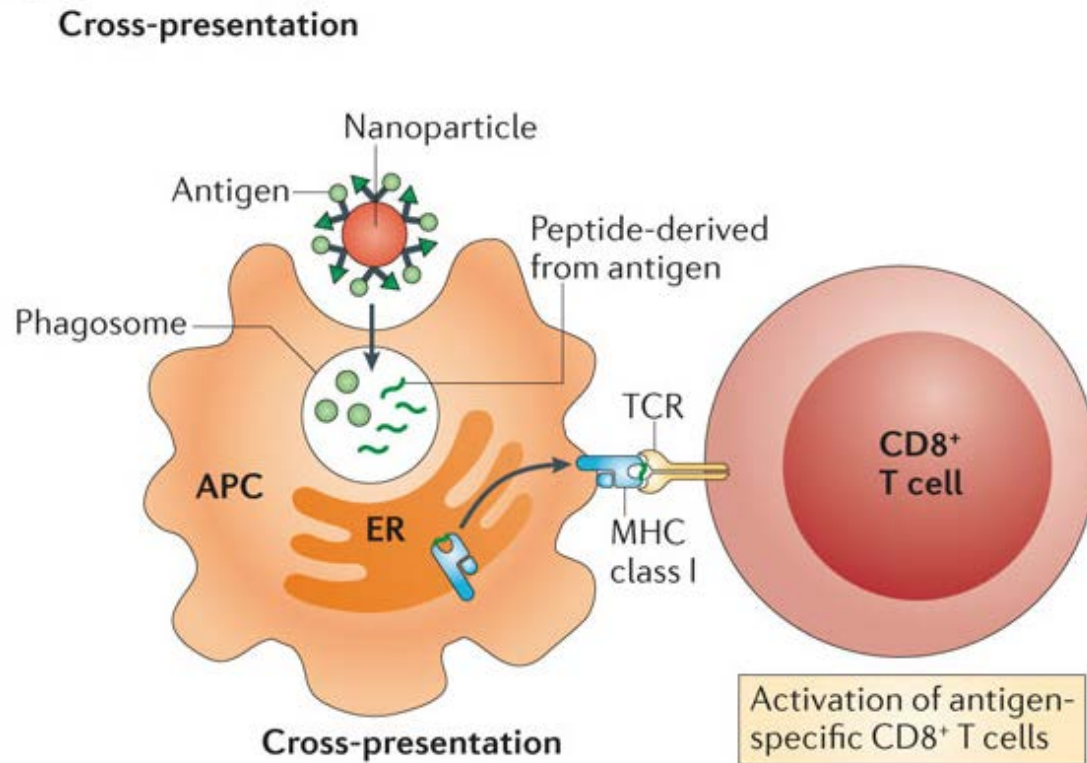
Virus-like particles, Dendrimers, etc

c Repetitive antigen display



Other matters

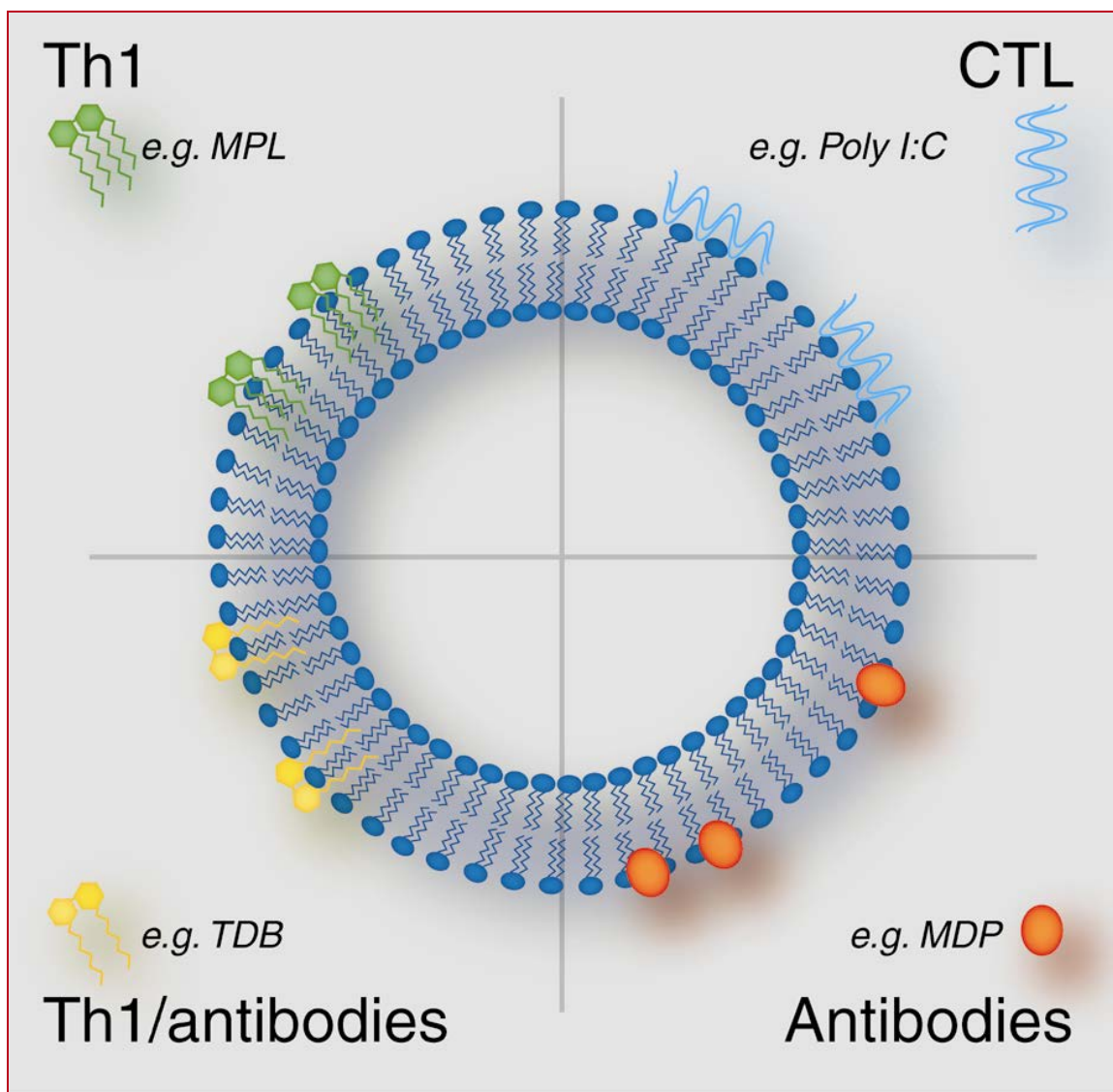
Cytosolic leakage of antigens/peptides for MHC class I cross-presentation
Co-delivery of cytokines, chemokines and immunomodulatory molecules



Smith et al. Nat Rev Immunol 2013 13: 701

SSI CAF01 adjuvant:

Cationic DDA liposome with TDB immunopotentiator/stabilizer



TDB (CAF01):

Trehalose dihebenate
TLR-independent activation
via MINCLE and IL-1

Poly (I:C):

Ds RNA
TLR3 agonist

CpG DNA

TLR9 agonist

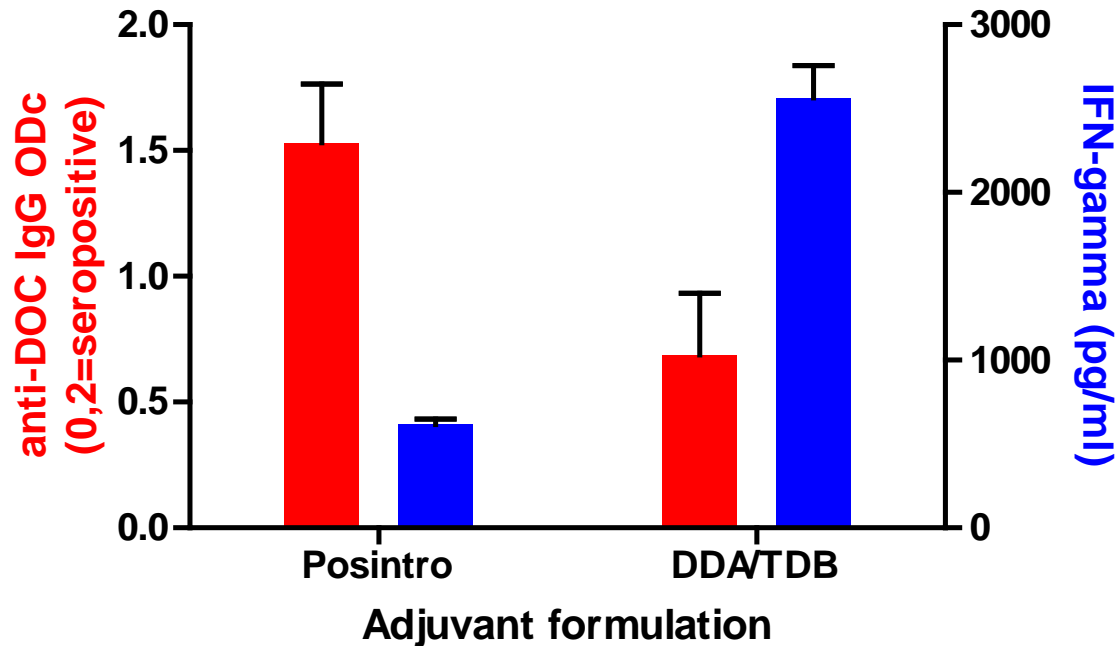
MPL:

Monophosphoryl lipid A
TLR4 agonist

MDP:

Muramyl dipeptide
NOD2 agonist

Adjuvants make a difference!



Mycoplasma hyosynoviae DOC-antigen with POSINTRO (Iscom) or DDA/TDB (CAF01) adjuvant 14 days after 2. vaccination

But still not so easy...

***Mycoplasma hyosynoviae* project 2007-2011**

10 different vaccine formulations (two antigens)

Three screenings for immunogenicity

Two best candidates in preclinical trial (CAF01)

Best candidate in clinical trial

200 vaccinated 200 control pigs

NO EFFECT on lameness, antibiotics or growth parameters

Den store ligning:

$$P_{ec} = \frac{AP+Sp-1}{Se+Sp-1} \int_a^b \epsilon \Theta + \Omega \int \delta e^{i\pi} = -1$$

$\sqrt{17}$
 ∞
 χ^2
 $\Sigma!$
 $\{2.7182818284\}$ θσέρτυθιοπσδφγηξκλ

Spørgsmål ?

