Westmead Millennium Institute
for Medical Research

Herpes simplex viral vaccine development

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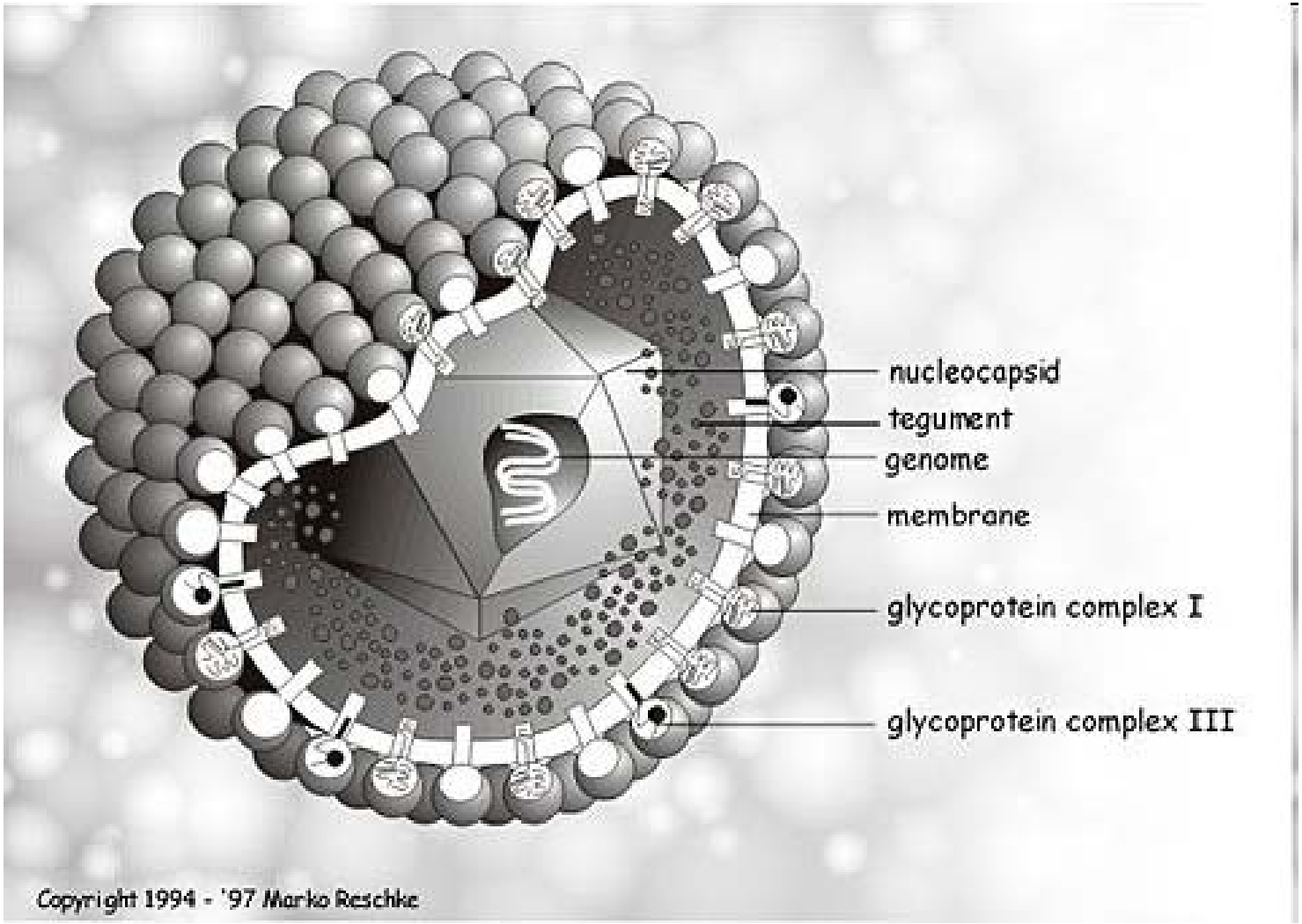
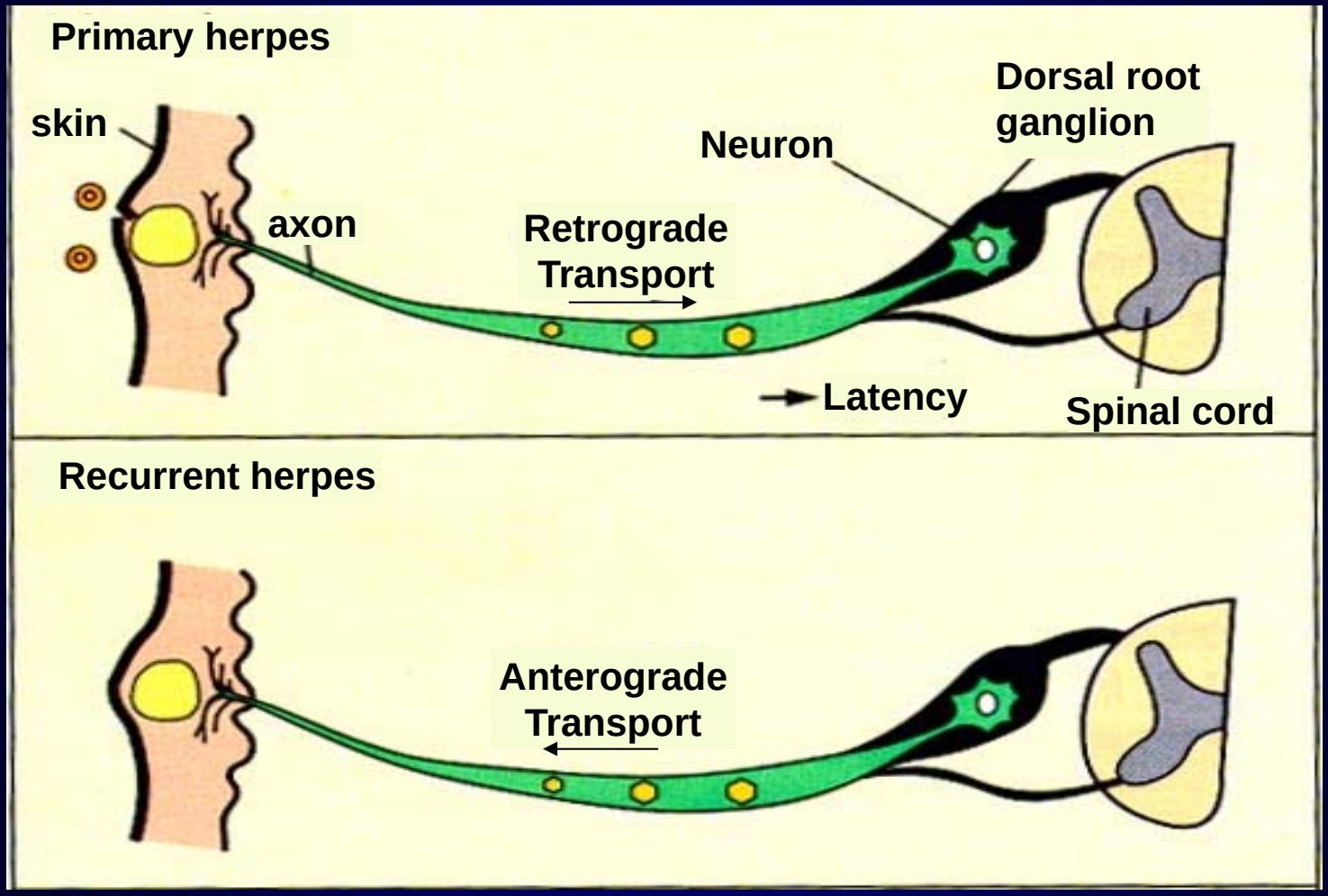
Herpesvirus structure

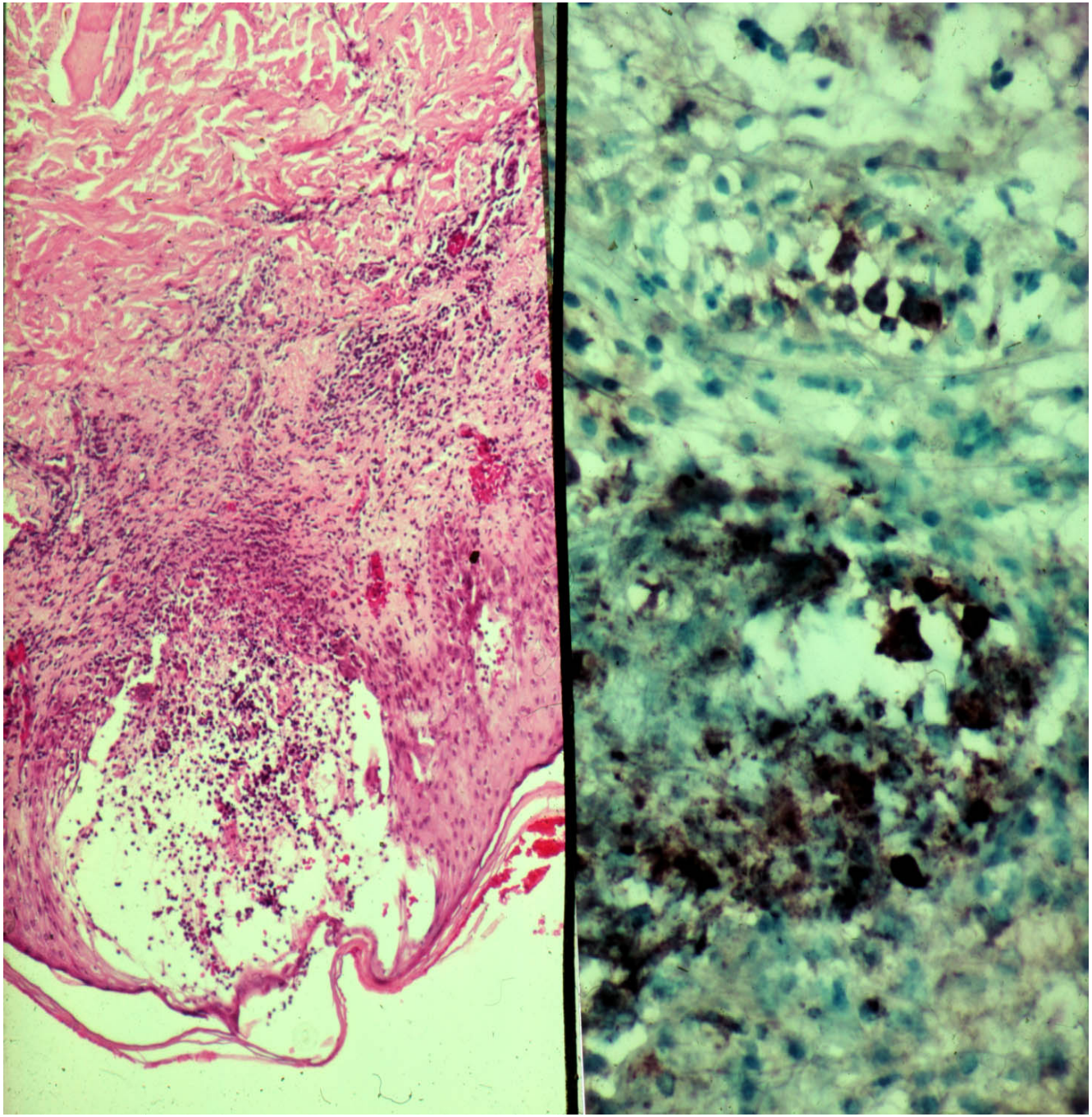


Epidemiology of HSV 1 and 2

- Worldwide: HSV1 seroprevalence 55% (USA) to >90% (Africa); HSV2 <10% (Japan) to 65%
- In Australia: 80% seropositive for HSV1, 12% for HSV2 (⇧ in indigenous, urban; Torres strait:50%)
- In developed nations, marked increase in HSV1 genital herpes in adolescents, young women
- Prior HSV2 (and HSV1 GH) infection increase risk of HIV acquisition 3-7 fold

Cycle of HSV infection





Immunopathogenesis of HSV infection and disease

- HSV1 and 2 closely related, many cross reactive and crossprotective antigens (to antibody and T cell)

- Immune control of HSV1/2 initial and recurrent infection at levels of both DRG and mucosa:

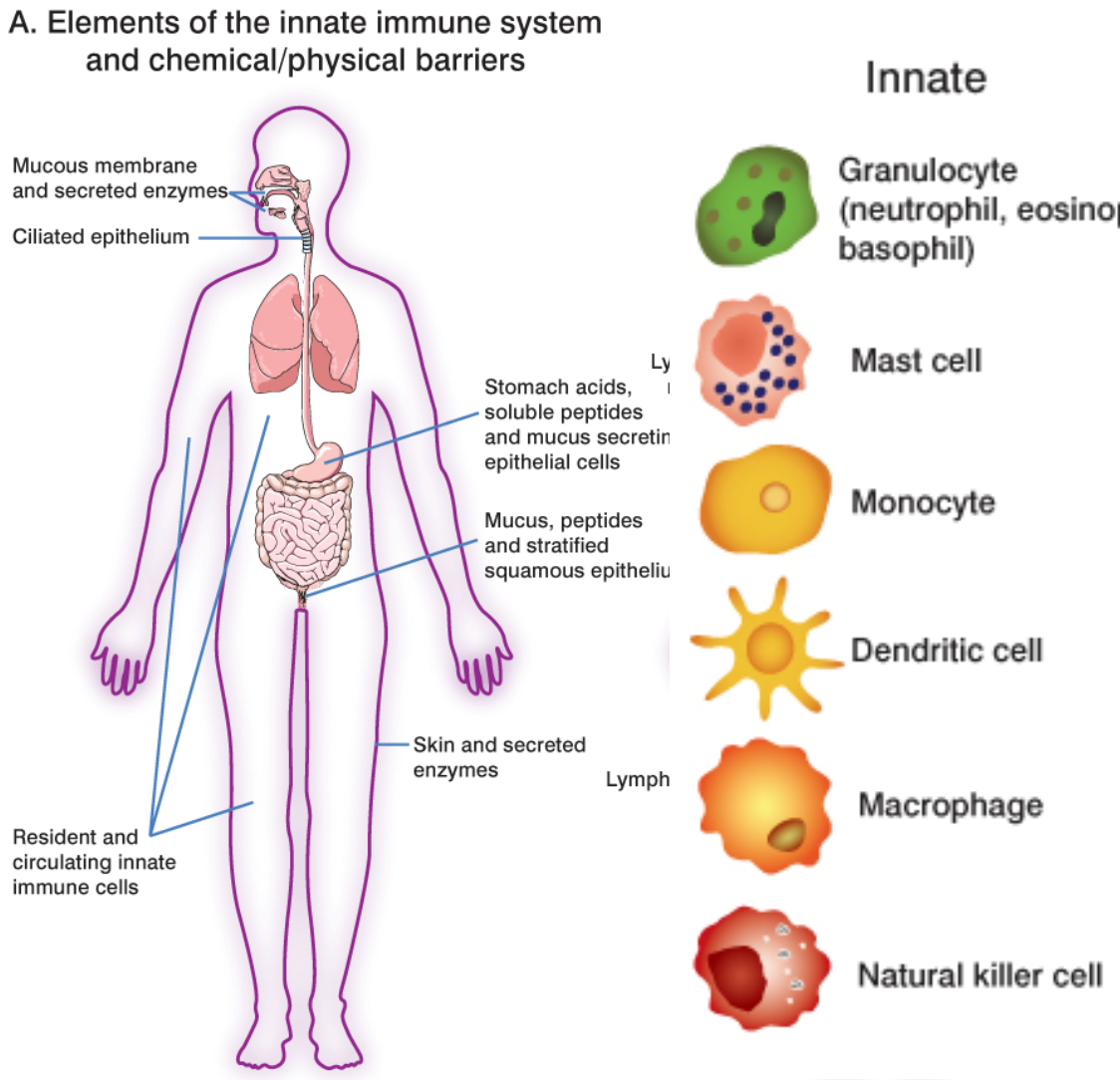
  - Innate; dendritic cells, interferon, NK cells, macrophages

  -Adaptive: neutralizing antibodies, CD4 and CD8 lymphocytes

- Genital HSV1 recurs infrequently

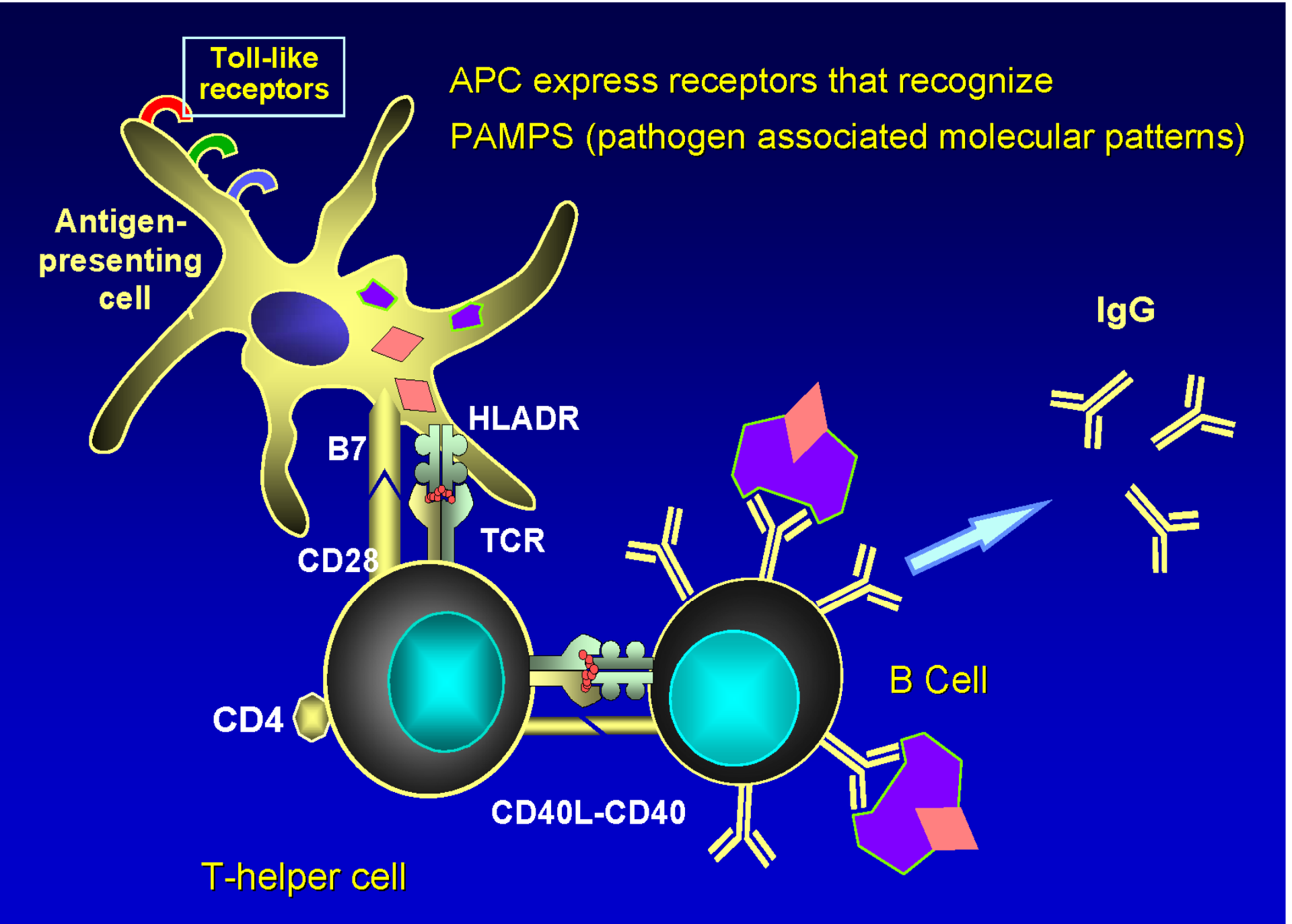
- Asymptomatic shedding of HSV1 and 2 at oral and genital mucosa respectively, HSV2 can be very frequent (12 hrly): responsible for most transmission

Components of innate immune system

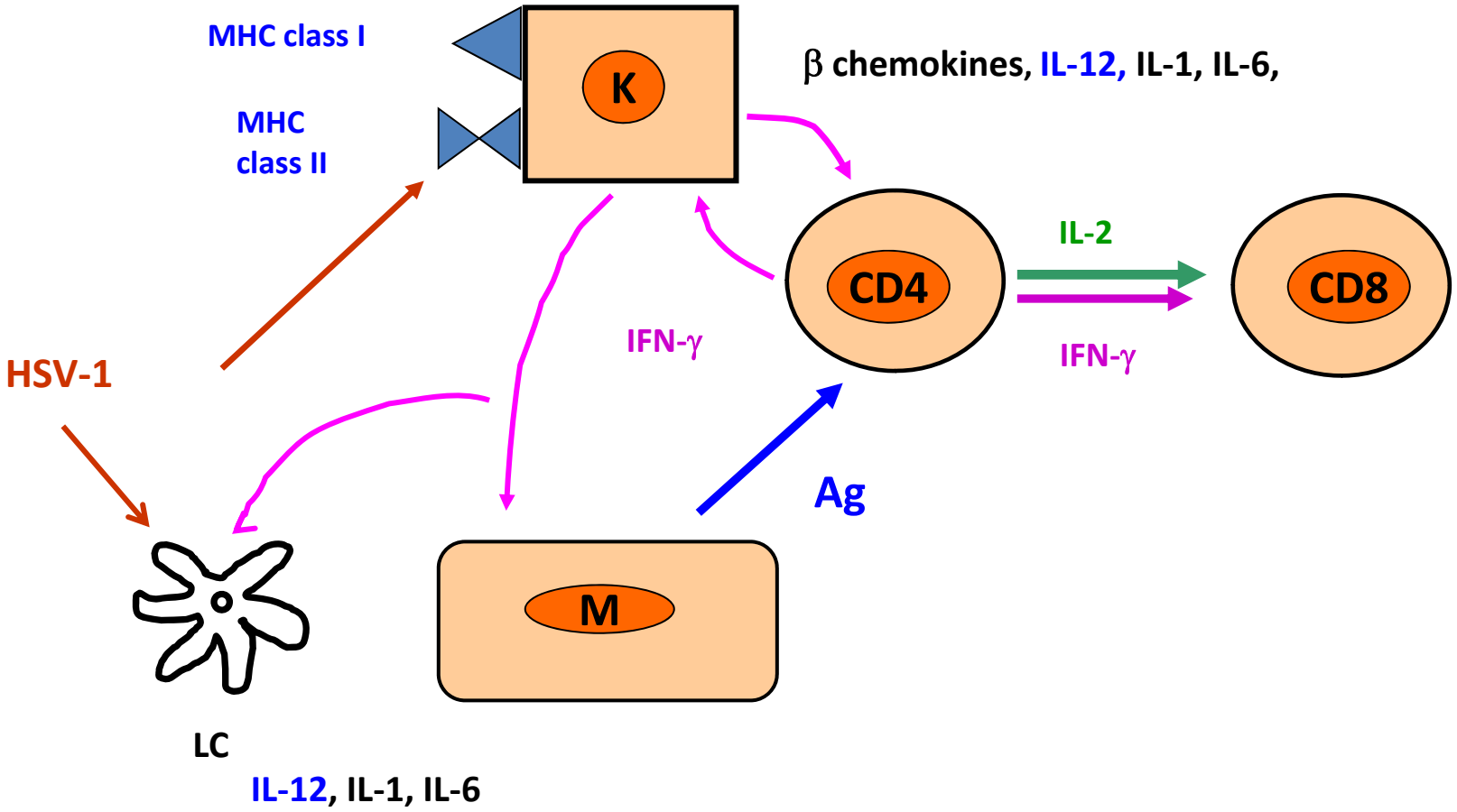


Chemokines

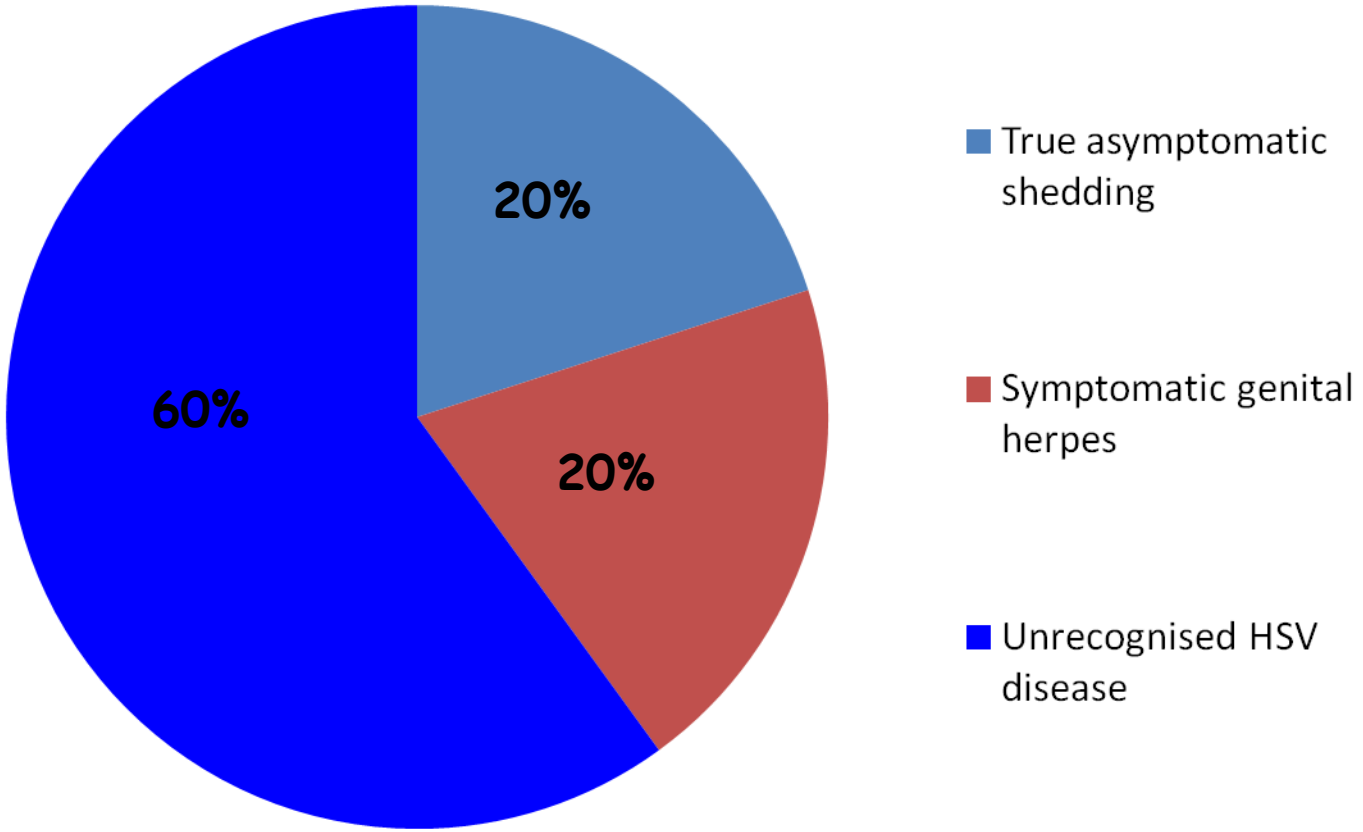
Cytokines


Toll-like receptors
APC express receptors that recognize
PAMPS (pathogen associated molecular patterns)
Antigen-presenting cell
IgG
HLADR
B7
TCR
CD28
CD4
B Cell
CD40L-CD40
T-helper cell

Role of immune cells and keratinocytes in lesions of recurrent Herpes simplex



Symptomatic vs asymptomatic Genital Herpes



Three ages of Immunization

- Paediatric
- Adolescence: Human Papillomavirus
  Herpes simplex virus*
  Epstein Barr virus*
  Hepatitis B virus
- Adult: Influenza virus
  Pneumococcus
  Zoster

AIMS of an HSV vaccine

- Reduce/eliminate viral replication in mucosa

- Prevent entry into nerves

  $\rightarrow \downarrow$ disease

  asymptomatic shedding

  - preventing infection (sterilizing immunity) too difficult?

- Now need vaccine for both genital HSV1 and 2

Challenges for developing Vaccines for Genital Herpes

- Latent HSV infection in neurones
  - normally express no MHCI
- HSV has mechanisms of evading the immune response
- Finding the best immunodominant protein stimulators/targets (large virus, ~80 proteins)
- Which are the most important immune mechanisms (cells)
  - How can they be best stimulated
- Delivery: DNA, recombinant virus, r-proteins
- Animal models

History of HSV vaccines

- Anderson and Burnet 1948: killed egg grown HSV1 for primary oral herpes in infants
- Many candidates since, including live attenuated (Roizman), killed, DNA extracted (Skinner), subunit: all failed
- 1999: Chiron vaccine recombinant HSV glycoproteins D and B: high neutralizing antibody titres but no efficacy against HSV2 acquisition
- GSK gD/dMPL: Simplirix trial 2002; Herpevac trial 2012

HSV2 gD/dMPL vaccine - the first (partially) successful vaccine for genital herpes

- Antigen: recombinant HSV2 glycoprotein D
- Adjuvant: ASO4 - Alum and monophosphoryl lipid A (DMPL)

  - Induces Th1 response (IFNg) in humans
- Simplirix trial: multicentre, RDBC

  - Consort design: immunize partners of subjects with GHD

Stanberry, Spruance, Cunningham et al NEJM 2002

Adjuvants

- Adjuvants are:

  - carriers (eg aluminium, emulsion) or

  - immunostimulatory molecules able to

  Modulate the immune response by

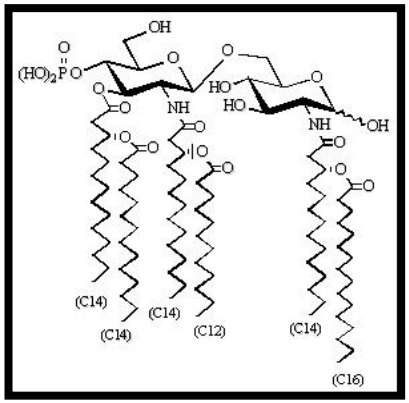
  - Activating dendritic cells

    (replace endogenous pathogen stimuli or PAMPs)
  - Stimulating the appropriate immune pathway

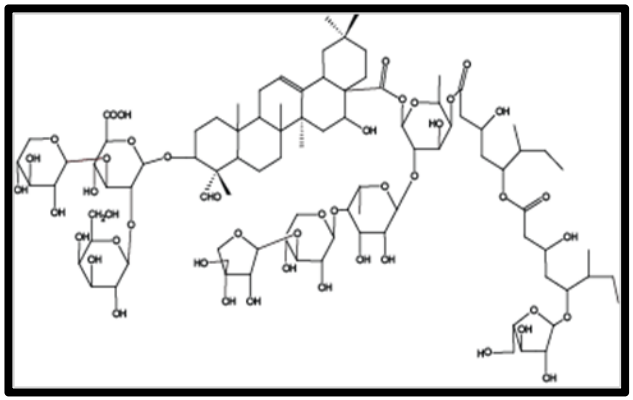
    → different patterns of cytokine production

Adjuvant Systems

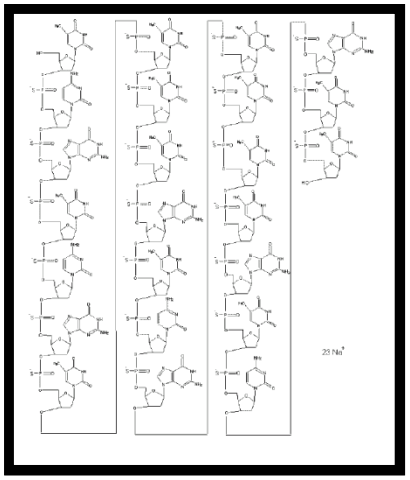
- Combinations of:
  - Classical adjuvants: aluminum salts, emulsion, liposomes
  - Immunostimulants: MPL, QS21, (CpG), tocopherol



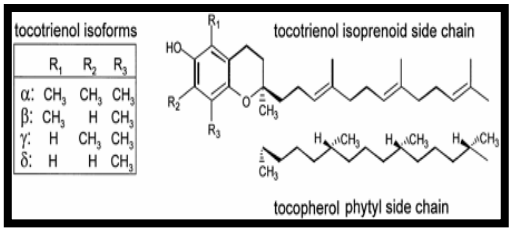
dMPL



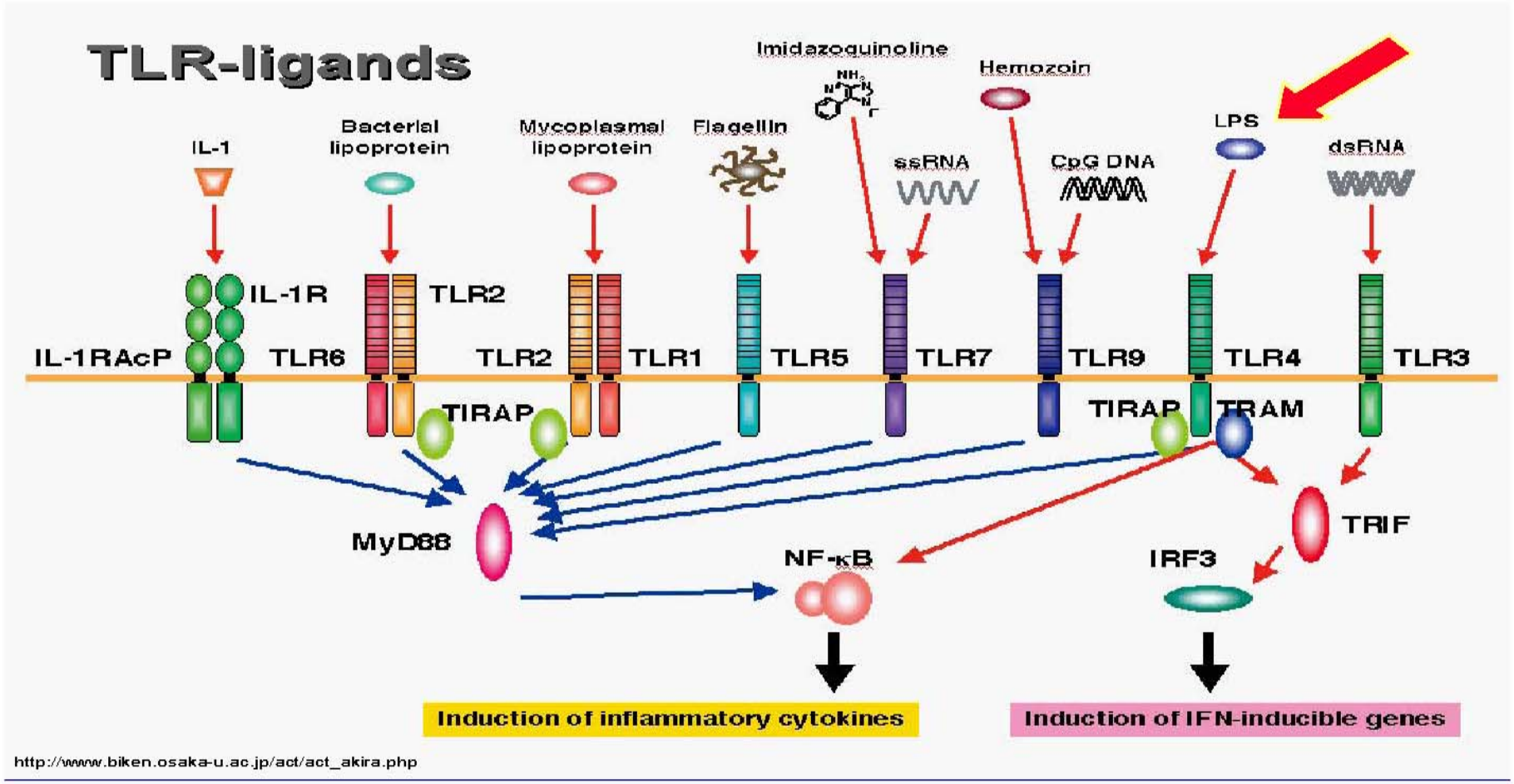
QS21



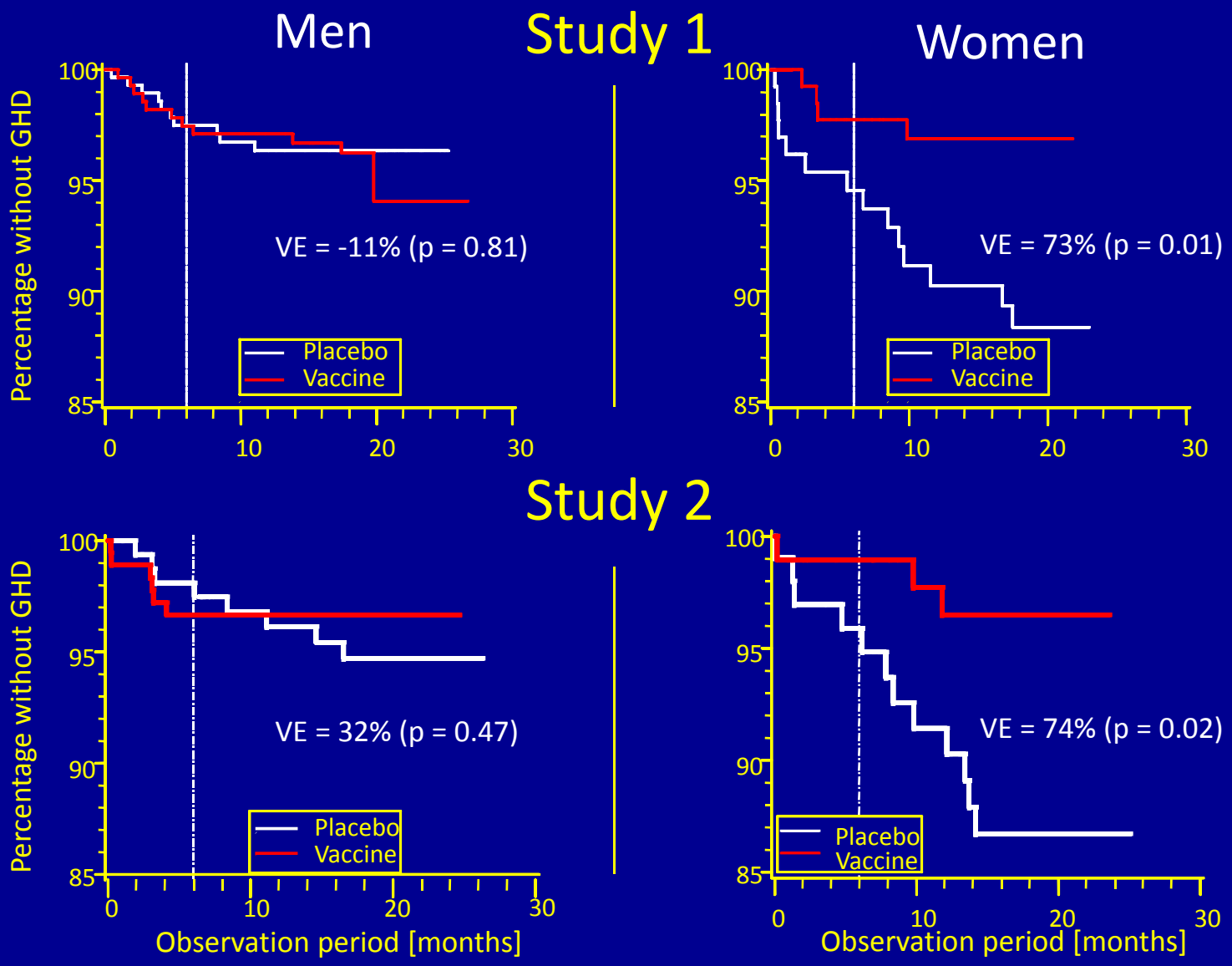
CpG



tocopherol


TLR-ligands
Imidazoquinoline
Hemozoin
IL-1
Bacterial lipoprotein
Mycoplasmal lipoprotein
Flagellin
ssRNA
CpG DNA
LPS
dsRNA
IL-1R
TLR2
IL-1RAcP
TLR6
TLR2
TLR1
TLR5
TLR7
TLR9
TLR4
TLR3
TIRAP
TIRAP
TRAM
MyD88
TRIF
NF-κB
IRF3
Induction of inflammatory cytokines
Induction of IFN-inducible genes
http://www.biken.osaka-u.ac.jp/act/act_akira.php

HSV2 gD vaccine prevents disease in female seronegative subjects



Lessons from the GSK GD-dMPL vaccine trial

- Why only efficacious in females?

  - gender specific general immunity?

  or

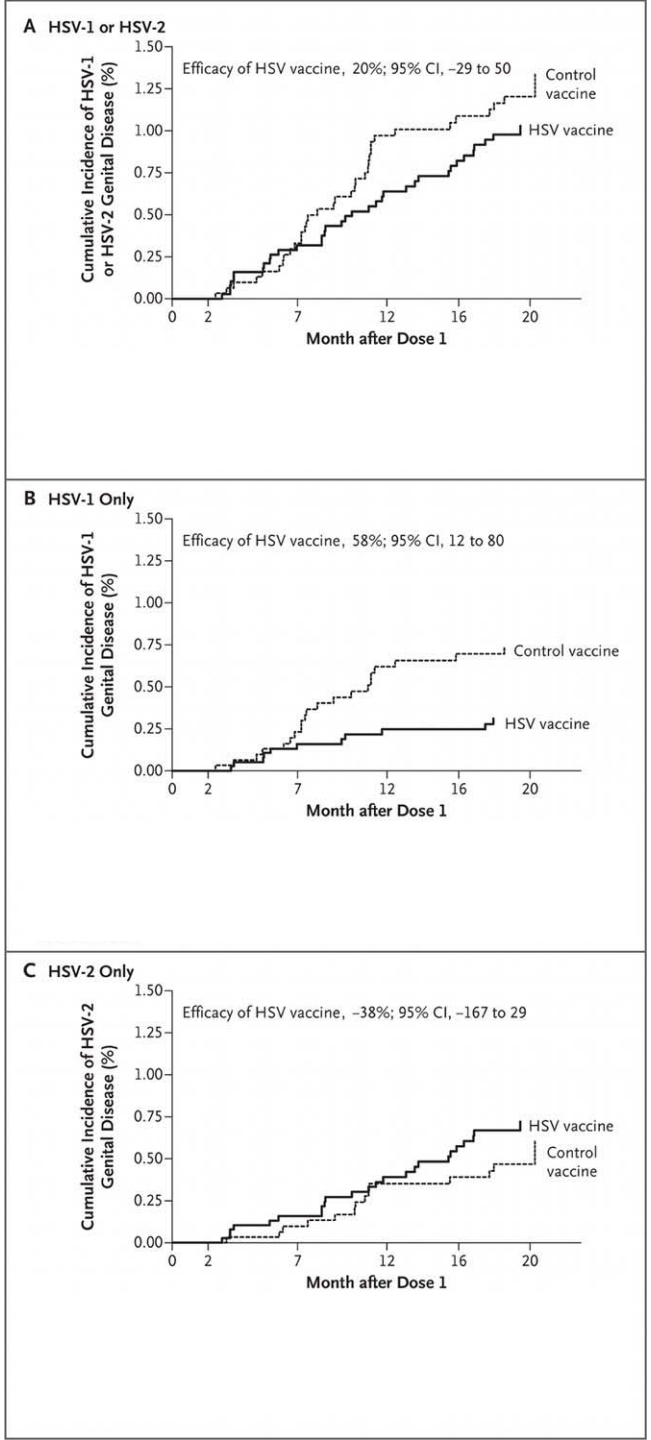
  - genital tract immunobiology

- What is the critical protective modality?

  Th1 cytokines induced by MPL

Herpevac trial 2012

- Random recruitment design
- HSV seronegative women, 18-30 years
- HSV1 predominant cause of genital herpes disease (GHD) in controls
- 58% efficacy against HSV1 GHD
- No efficacy against HSV2 GHD


A HSV-1 or HSV-2
Efficacy of HSV vaccine, 20%; 95% CI, −29 to 50
Control vaccine
HSV vaccine
Cumulative Incidence of HSV-1 or HSV-2 Genital Disease (%)
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 2 7 12 16 20
Month after Dose 1
B HSV-1 Only
Efficacy of HSV vaccine, 58%; 95% CI, 12 to 80
Control vaccine
HSV vaccine
Cumulative Incidence of HSV-1 Genital Disease (%)
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 2 7 12 16 20
Month after Dose 1
C HSV-2 Only
Efficacy of HSV vaccine, −38%; 95% CI, −167 to 29
HSV vaccine
Control vaccine
Cumulative Incidence of HSV-2 Genital Disease (%)
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 2 7 12 16 20
Month after Dose 1

Why such differences between the Herpevac and Simplirix Trials

- Cohorts:

  - Simplirix: women with partners with GHD = ?Mucosal priming vs none with Herpevac

- Why efficacy with HSV1 and not HSV2: ?Easier to obtain immune control, much cross protection/immunogenicity

  - via antibody and T cells

HSV vaccines: current issues

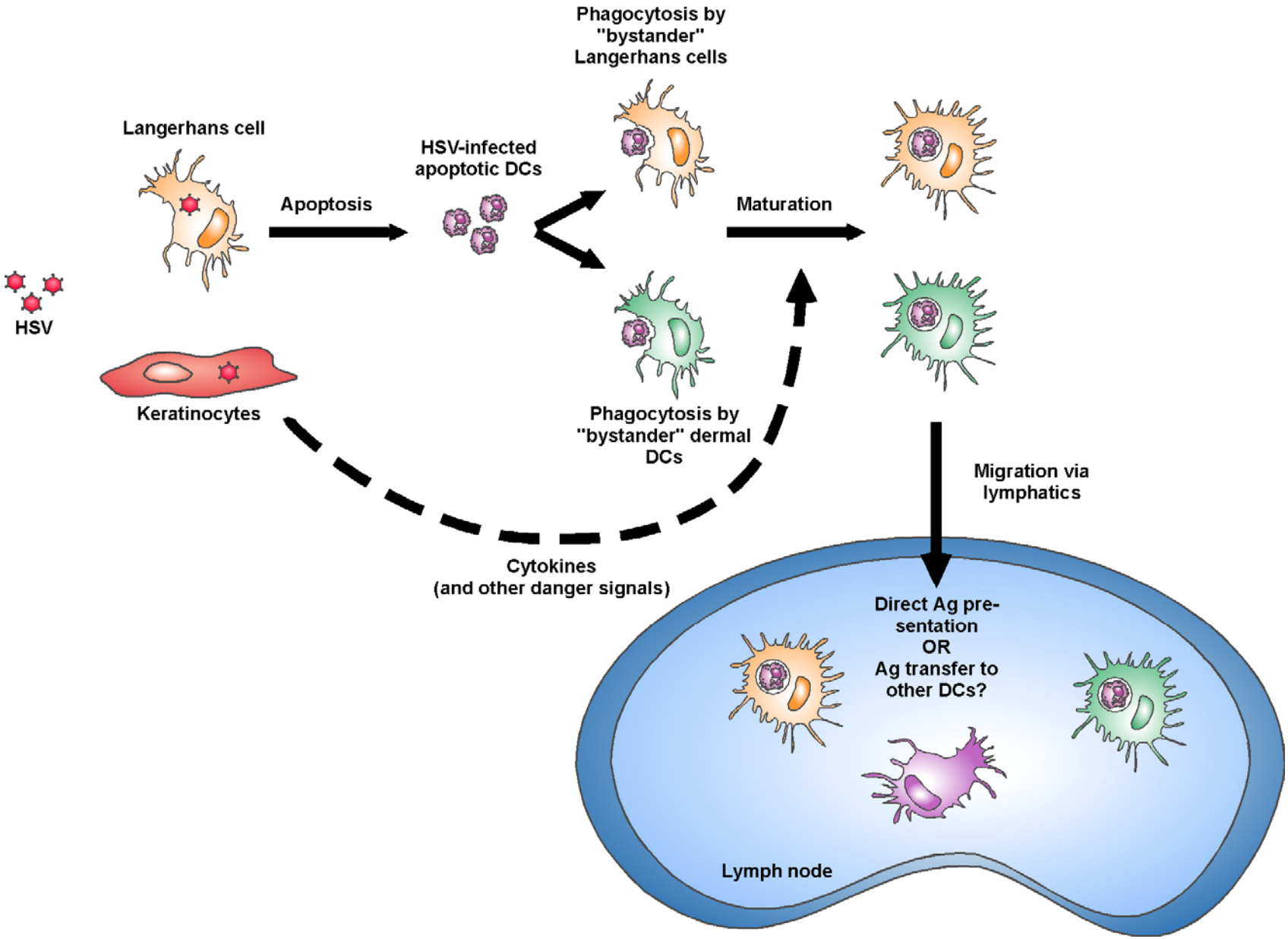
- Priority:in developed vs developing world
- Need vaccine for HSV1 and 2?
- What immune modalities required to stimulate: Neut antibody, CD4/CD4 lymphocytes, Dendritic cells, other aspects of innate immunity
- Need better animal models to help decide
- Need human CD8 lymphocyte adjuvant
- What are best candidates: specific live attenuated vaccines, viral vector, recombinant proteins

Current classification of vaccines

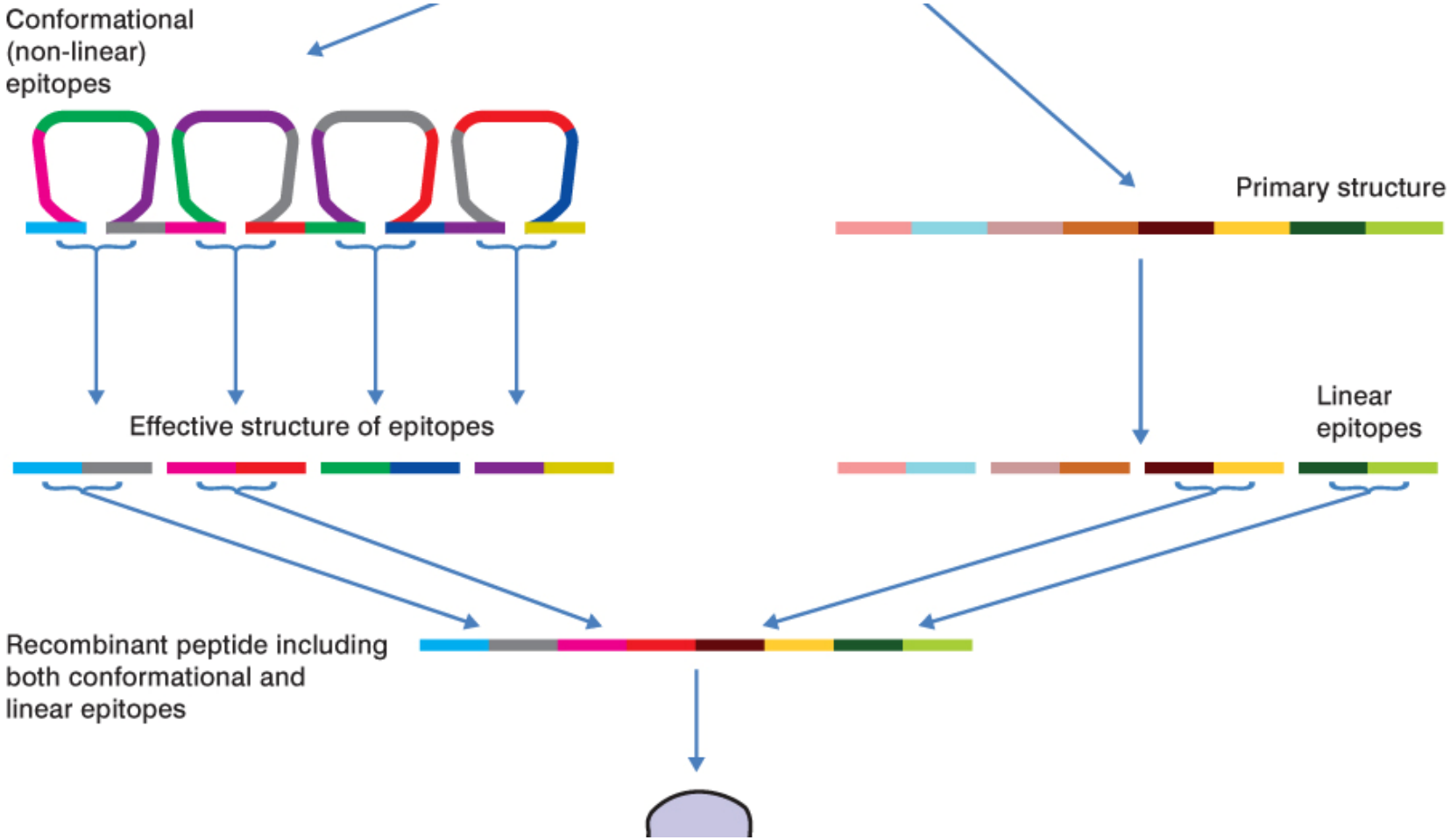
- Live attenuated (mumps, measles)
- Inactivated or killed (HAV)
- Subunit (influenza)
- Polysaccharide conjugated (H, Influenza, Pneumococcal)
- Virus Like Particles
- DNA
- Live vectors
- Subunit or recombinant antigens (including peptides) with Adjuvant

Advantages and disadvantages of live attenuated versus killed vaccines

Live, attenuated	Killed/inactivated
Eg OPV, MMR, VZV, some influenza, BCG	Eg IPV, HAV, whole-cell pertussis
Mimic the natural infection, may retain immune evasion factors	Usually require adjuvants
Strong priming (1–2 doses)	Multiple doses needed for priming
Long-term persistence of immunity	Booster may be needed for long-term immunity
May induce mild disease symptoms	No disease symptoms
Rare reversion to virulence	No reactivation, non-infectious
Potential for immunological interference with other live vaccines	Low risk of immunological interference
Less stable over time	More stable over time
Poor resistance to cold chain deviation	Better resistance to cold chain deviation
Affected by administration of blood/blood-derived products or maternal antibodies in infants	Generally not affected by administration of blood/blood products

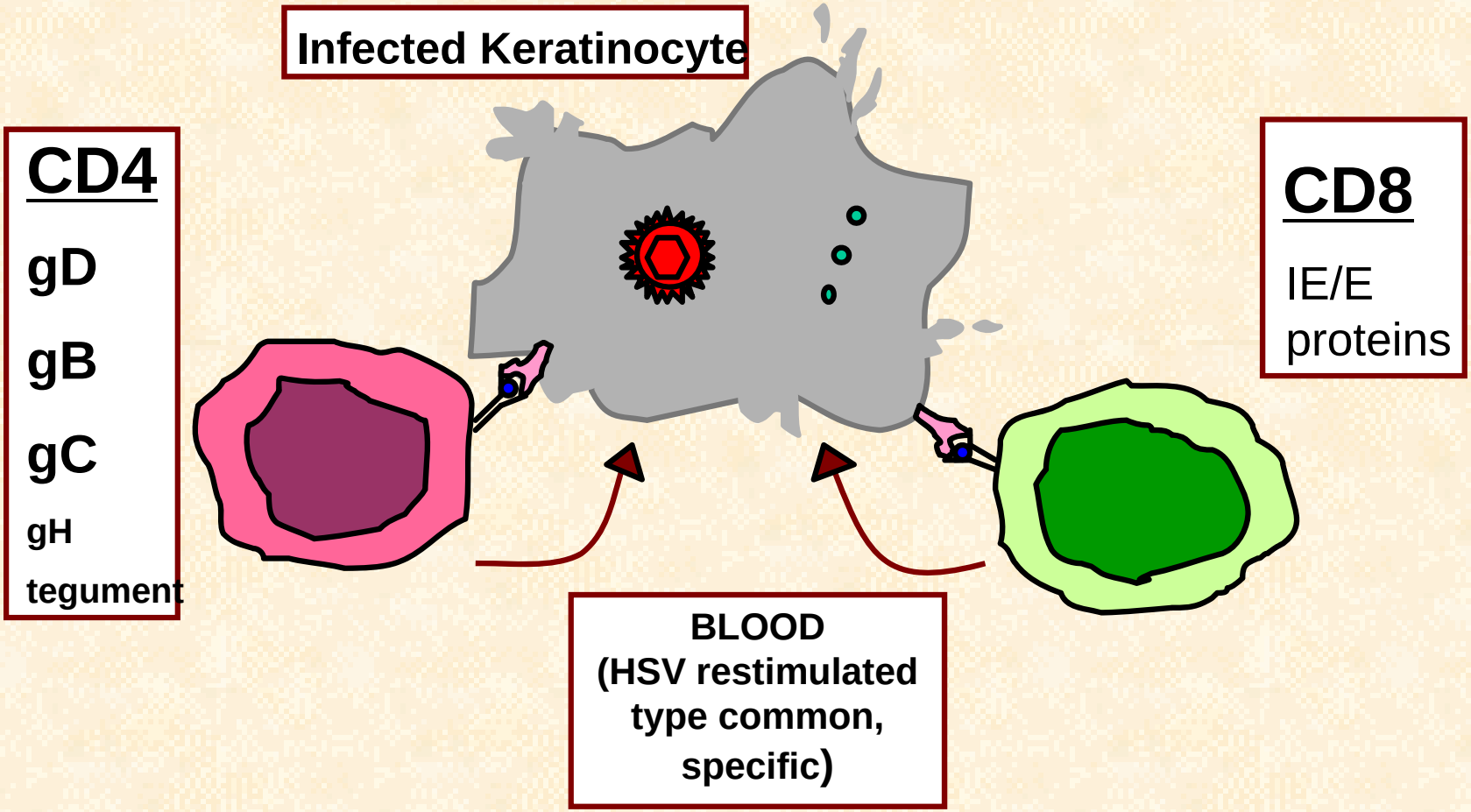

Phagocytosis by "bystander" Langerhans cells
Langerhans cell
HSV-infected apoptotic DCs
Apoptosis
Maturation
HSV
Keratinocytes
Phagocytosis by "bystander" dermal DCs
Migration via lymphatics
Cytokines (and other danger signals)
Direct Ag pre-sentation OR Ag transfer to other DCs?
Lymph node

New approaches: Synthetic peptide antigens

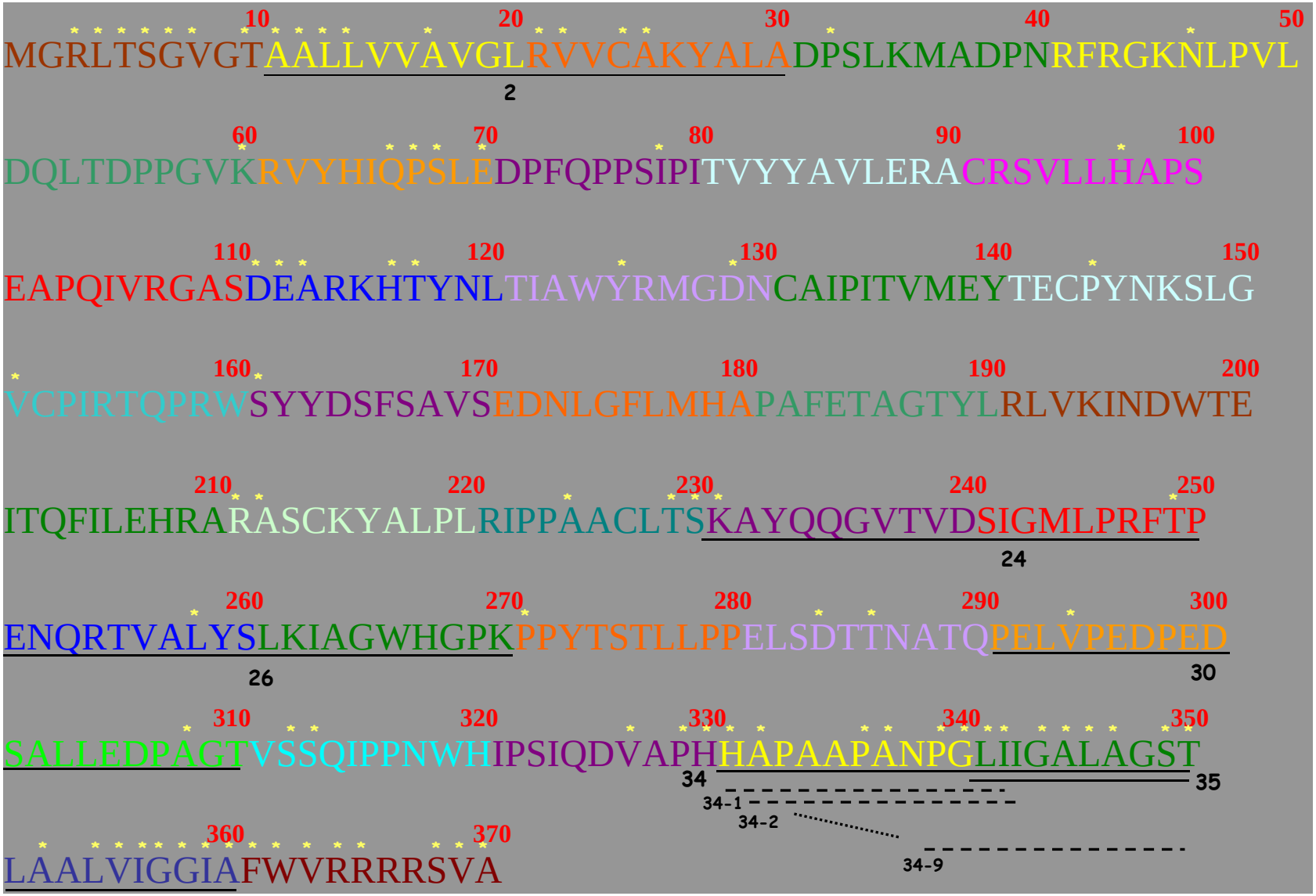


HSV Vaccine Candidates

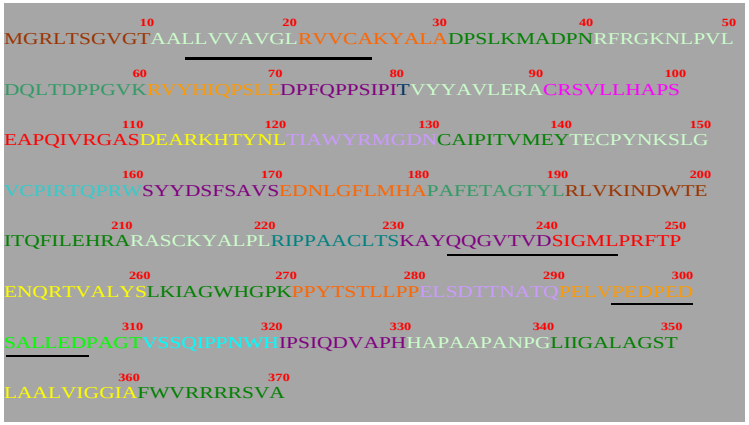
HSV1/2 protein targets for CD4 and CD8 lymphocytes



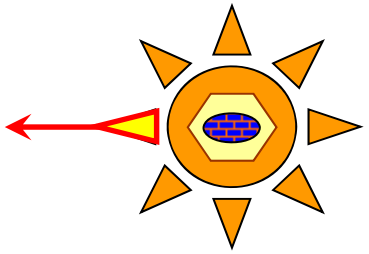
HSV-2 gD (gD2) Sequence

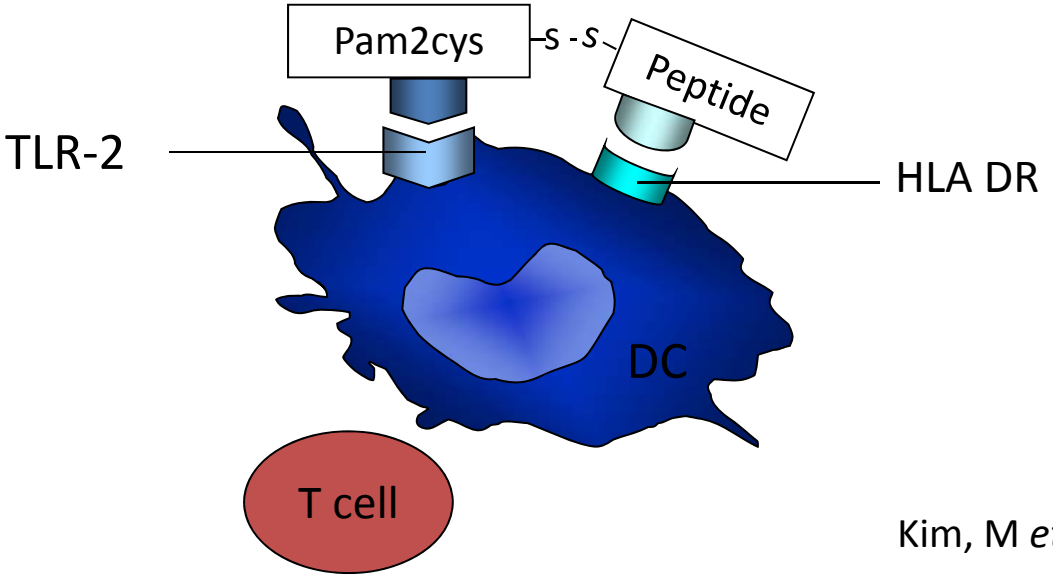


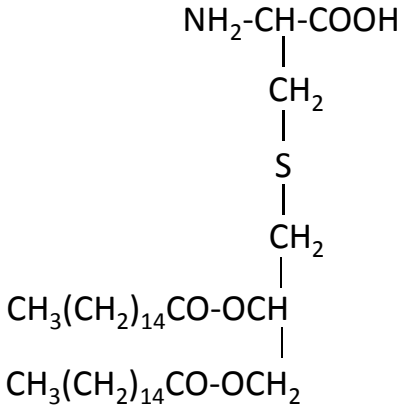
HSV2 Peptide-TLR2 stimulant vaccine



Glycoprotein D of HSV-2 (gD2) for CD4 T cell



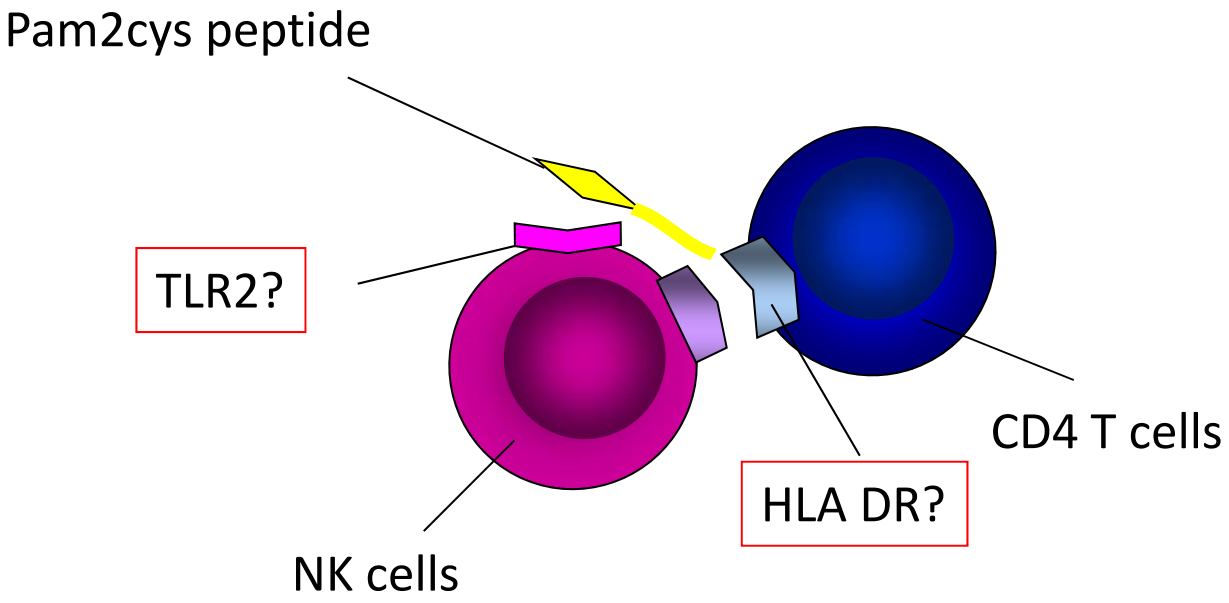




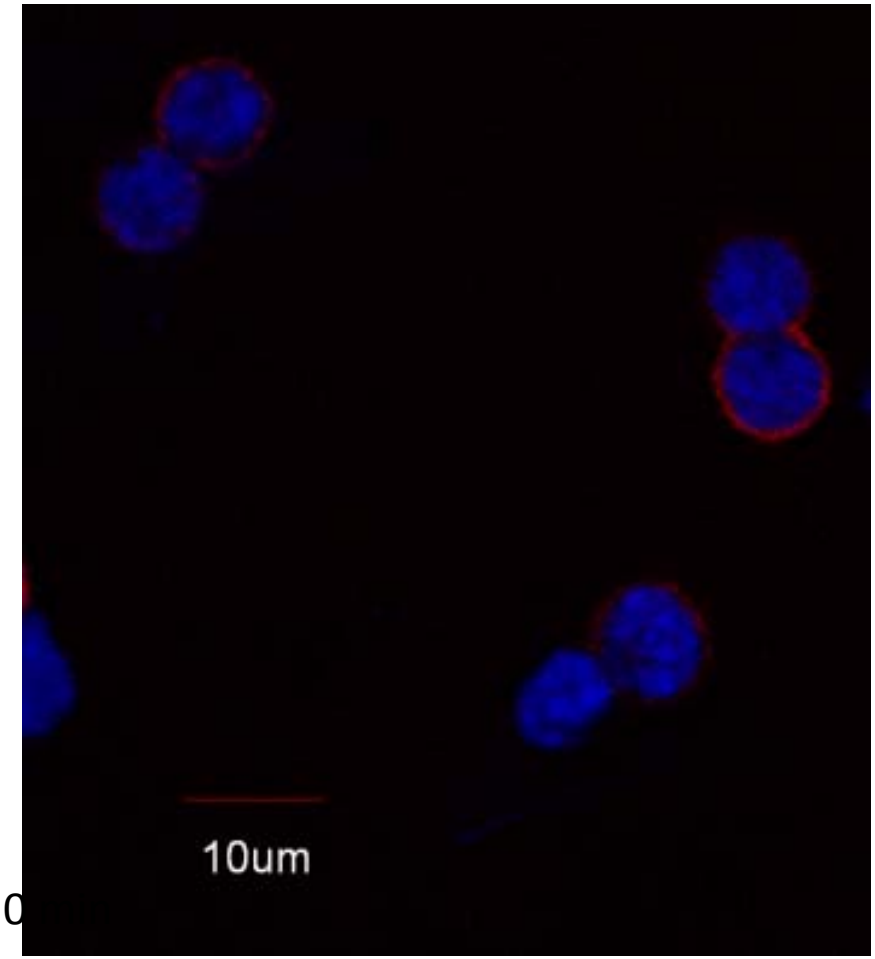
Kim, M *et al.*, *J Immunol*. 181, 2008

- gD2 lipopeptide stimulates NK cells as well as Dendritic cells via TLR2 which they both express

- How do NK and CD4 T cells interact with each other? Cytokines vs direct contact



NK cell-CD4 lymphocyte conjugates induced by lipopeptide



Conclusions

- Triple DC, NK and CD4 T cell interaction is required for maximal stimulation of PBMC by lipopeptide.
- Act in pairs: DC-NK, DC-CD4, NK-DC and as a trio

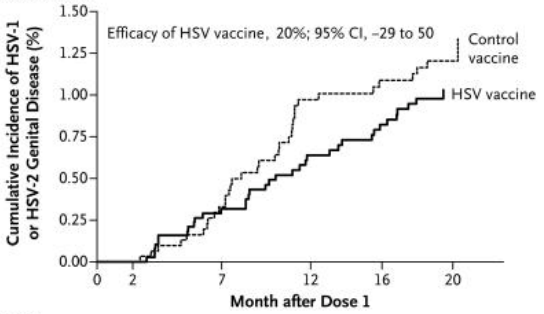
  - interacting via ‘immunologic’ synapses and cytokines

- Must consider NK cells as well as DCs as targets for adjuvants

Vaccines for Genital (and neonatal) Herpes: Conclusions

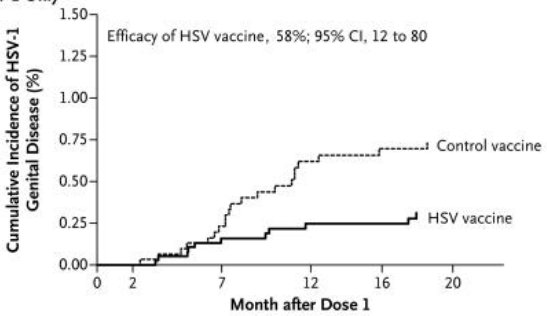
- Better knowledge of mechanisms of immune control and antigen presentation will allow most appropriate selection of adjuvants
- Like HIV, HSV vaccine should be aimed at all immune modalities - innate and adaptive:, myeloid & plasmacytoid DCs & NK cells, CD4 and CD8 lymphocytes, neutralizing antibodies
- Need HSV1/2 cross reactive vaccine
  - Increasing incidence of HSV-1 genital herpes in adolescence
- Many vaccine candidates, incuding specific live attenuated, DNA, killed and recombinant protein and viral vector vaccines will be trialled in the future
- Is there a place for peptide-adjuvant vaccines - Alone, in combiation or just to elucidate appropriate antigens and adjuvants?

A HSV-1 or HSV-2



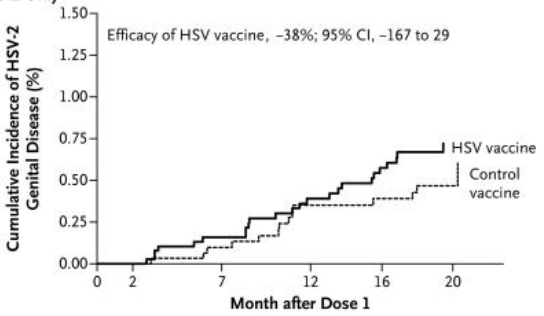
No. at Risk						
HSV vaccine	3798	3632	3332	3211	1186	
Control vaccine	3076	2948	2666	2560	963	
Cumulative No. of Events						**Total**
HSV vaccine	0	12	23	29	35	35
Control vaccine	0	10	28	31	34	35

B HSV-1 Only



No. at Risk						
HSV vaccine	3798	3637	3345	3229	1192	
Control vaccine	3076	2950	2675	2570	969	
Cumulative No. of Events						**Total**
HSV vaccine	0	6	9	9	11	11
Control vaccine	0	7	18	20	21	21

C HSV-2 Only



No. at Risk						
HSV vaccine	3798	3638	3340	3219	1190	
Control vaccine	3076	2955	2684	2580	971	
Cumulative No. of Events						**Total**
HSV vaccine	0	6	14	20	24	24
Control vaccine	0	3	10	11	13	14

Key Remaining Vaccine challenges

Pathogens

- Highly variable pathogens that evade the immune system
  - HCV, HIV, TB, Herpesviruses
- Pathogens requiring multistage immune responses
  - Malaria
- Safer immunogens are potentially weak
  - Highly purified or recombinant proteins/peptides
  - Polysaccharides
- Need for rapid immunity
- Need for cross protection against antigenic variants
  - Pandemic influenza subtypes

Classes of Licensed Adjuvants

Only a few adjuvants are used in registered commercial vaccines for human use:

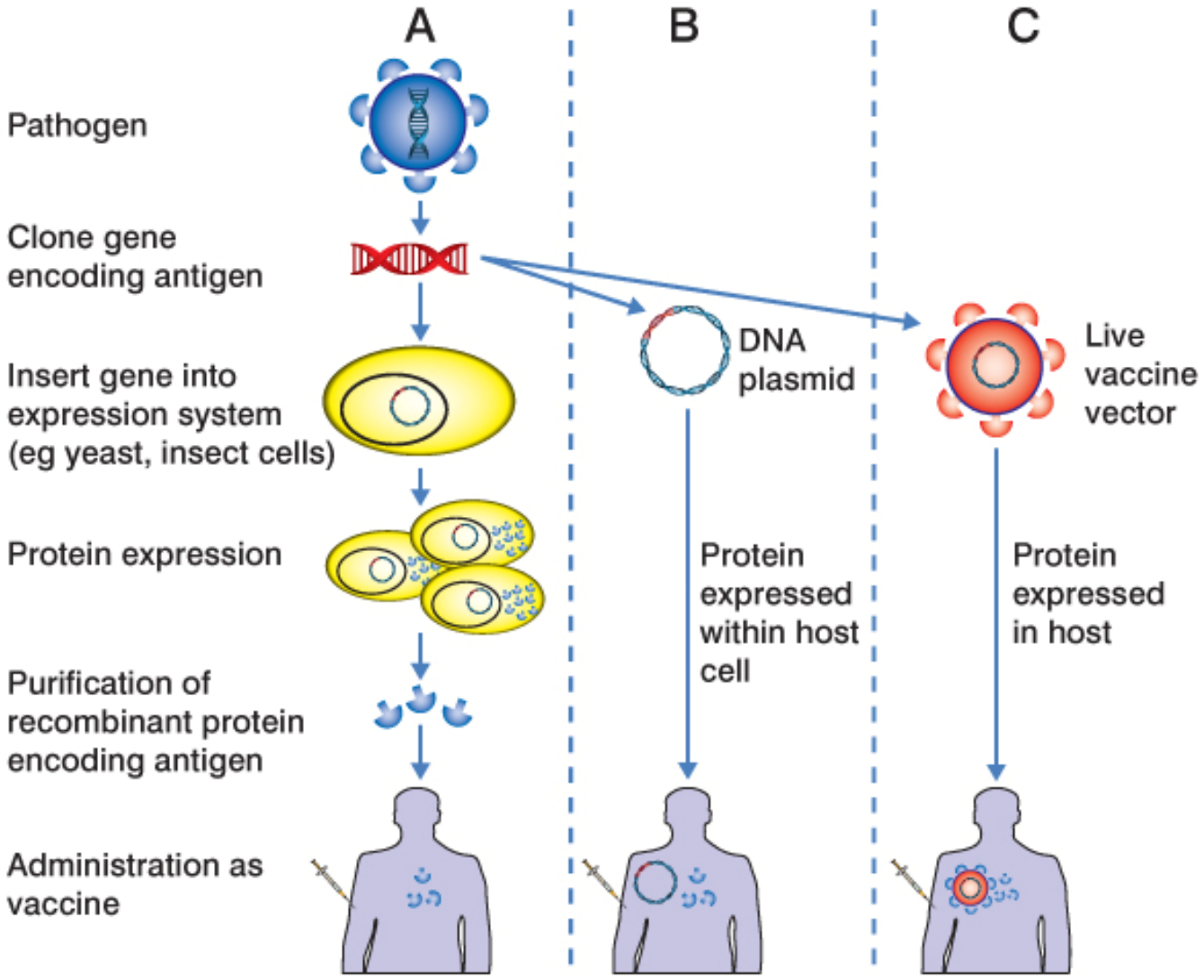
- Aluminum salts: the most widely used adjuvant
- Emulsions: MF59 (Novartis)
- dMPL + Aluminum：AS04 (GSK Bio)

Why we need NEW adjuvants

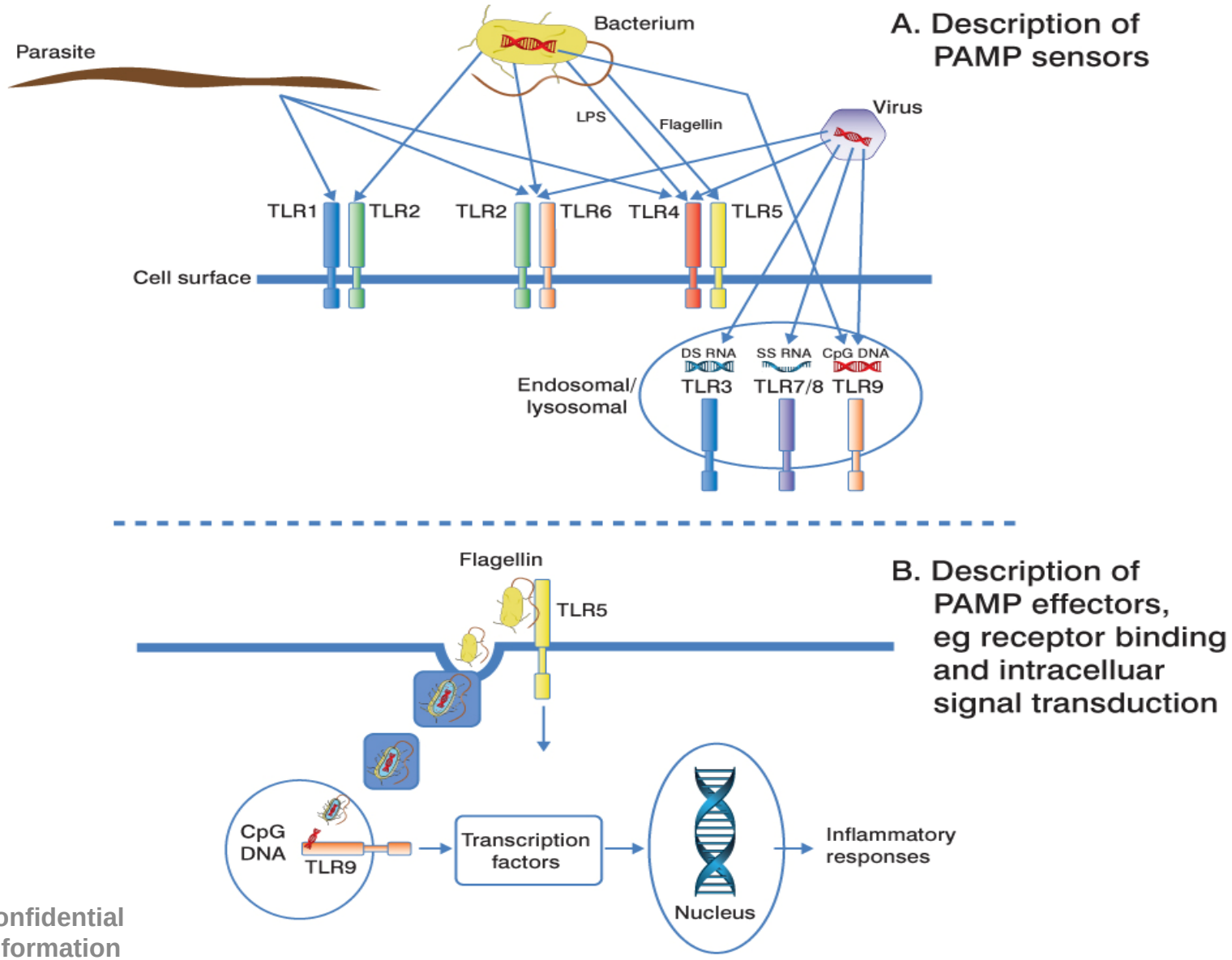
- To better target immune responses (humoral and cellular, Th1, CD8)
- To induce higher and longer-term persistence of protection
- To bypass weakened immunity:
  - Immunosenescence
  - Immunosuppression
- To reduce the amount of antigen needed (antigen-sparing effect eg pandemic influenza)
  - Need for the right adjuvant(s) with the right antigen(s) to protect against disease in the right target population (ie tailoring)

Identifying and producing vaccine antigens (2)

- Recombinant protein Ag
- DNA
- Live vaccine vector

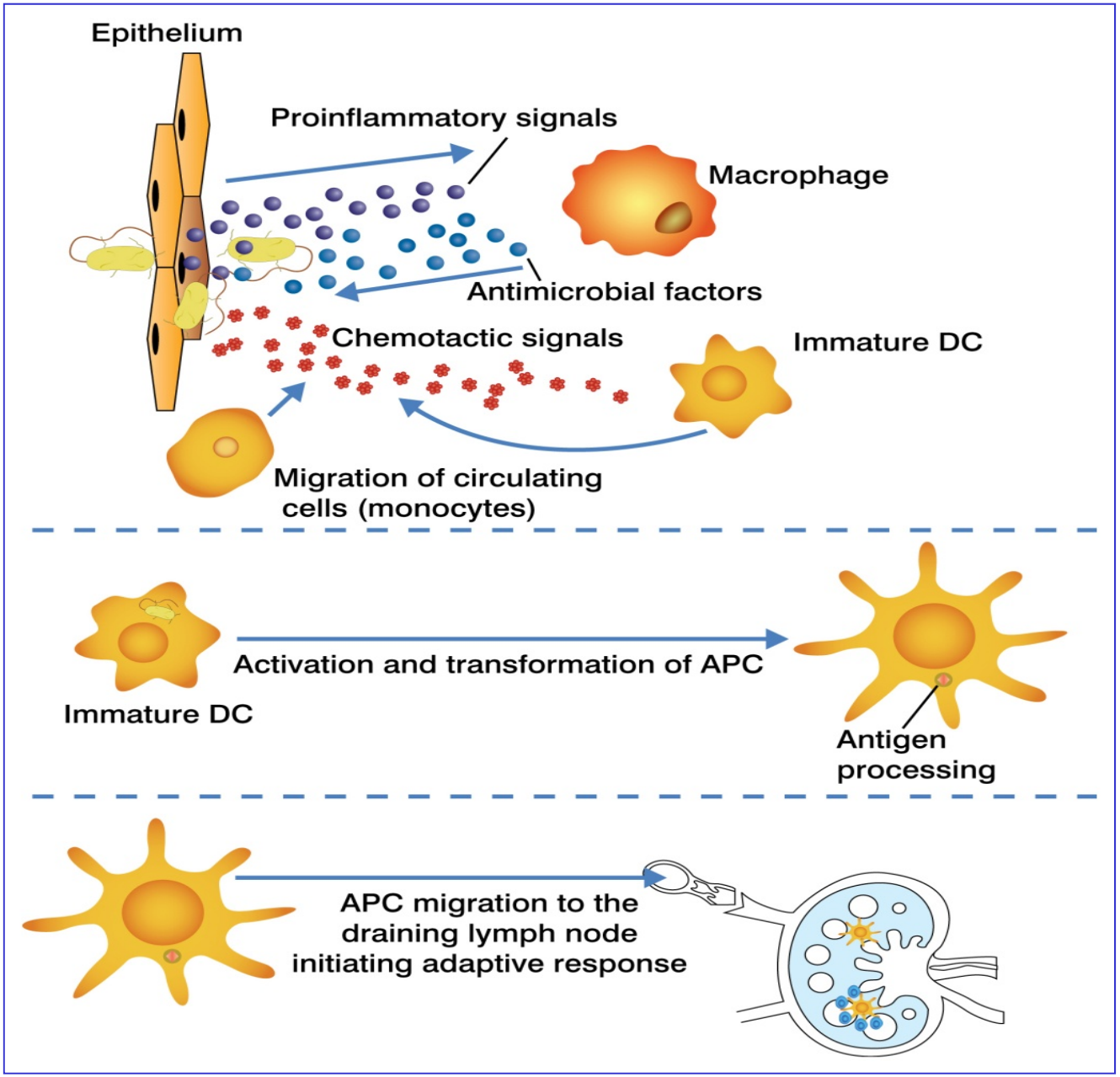


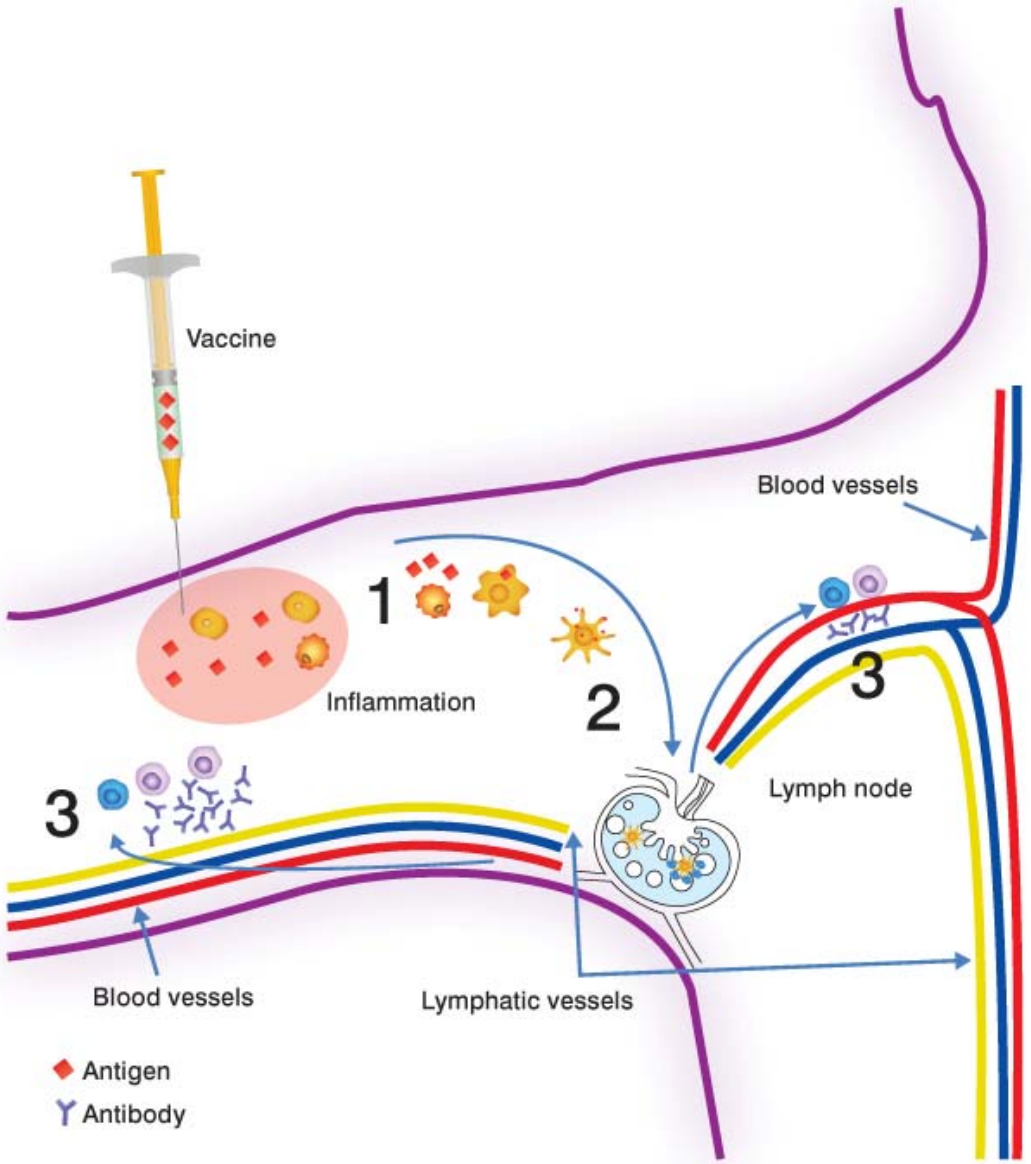
Early detection and response to pathogens



Detection of pathogens by innate immune cells

APCs translate and drives information to the adaptive immune system





Vaccine composition

Correct target / stimulator :

viral protein or gene

- Correct immune mechanism:

adjuvants, cytokines

Results of Simplirix trial 2002 (GSK gD2 dMPL vaccine)

In HSV1-2- females

Vaccine efficacy	**STUDY 1**	**STUDY 2**
Disease	73% (p=0.01)	74% (p=0.02)
Infection	48% (p=0.06)	39% (p=0.07)

Males

HSV1+2- females] no efficacy

Mild moderate local reactogenicity

Simplirux trial of GSKgD/dMPL vaccine, 2002: Conundrums

Why only efficacious in women

- gender specific general immunity?

Or

- genital tract immunobiology

- Why no enhanced effect in HSV1+2- females (constantly re-immunised by HSV1?)
- Is there really protection against infection? p=0.06
- What was the critical protective modality
  - ? no CTLs
  - Th1 cytokines induced by dMPL not MF59 (?IFNγ)

Herpes Simplex Vaccines

Improvements

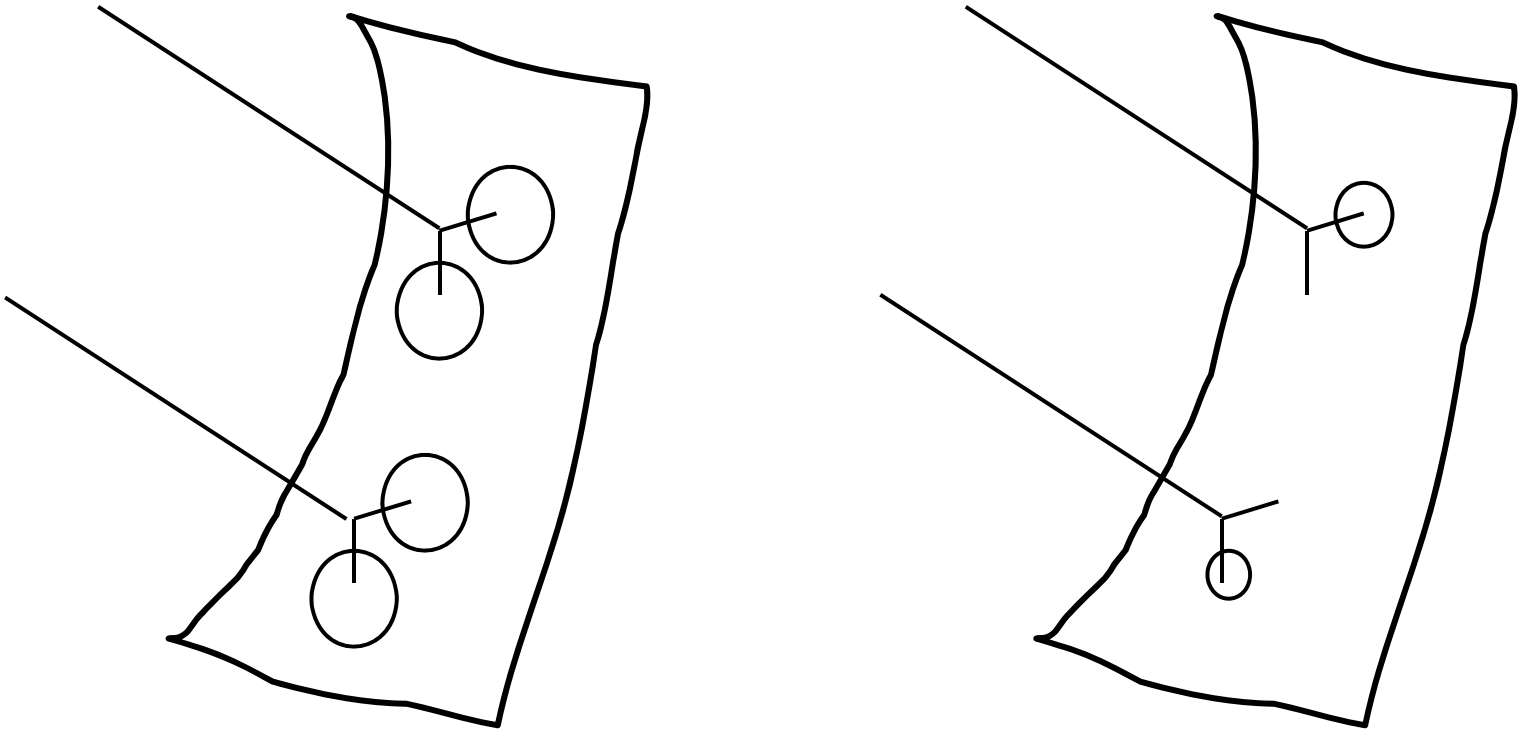
1) To recombinant protein vaccine

- Broader range of targets/stimulators (eg ICP27)
- Induce T-lymphocyte cytotoxicity (adjuvant like QS21)

2) Alternate strategies

- DNA vaccines
- Recombinant viruses: vaccinia, avipox
  - incorporate viral antigens, cytokines
- Mucosalvaccines

Neutralizing anti-gD and interferons inhibit axonal transmission of HSV and spread in ECs (Mikloska et al JVI 1999,2001)

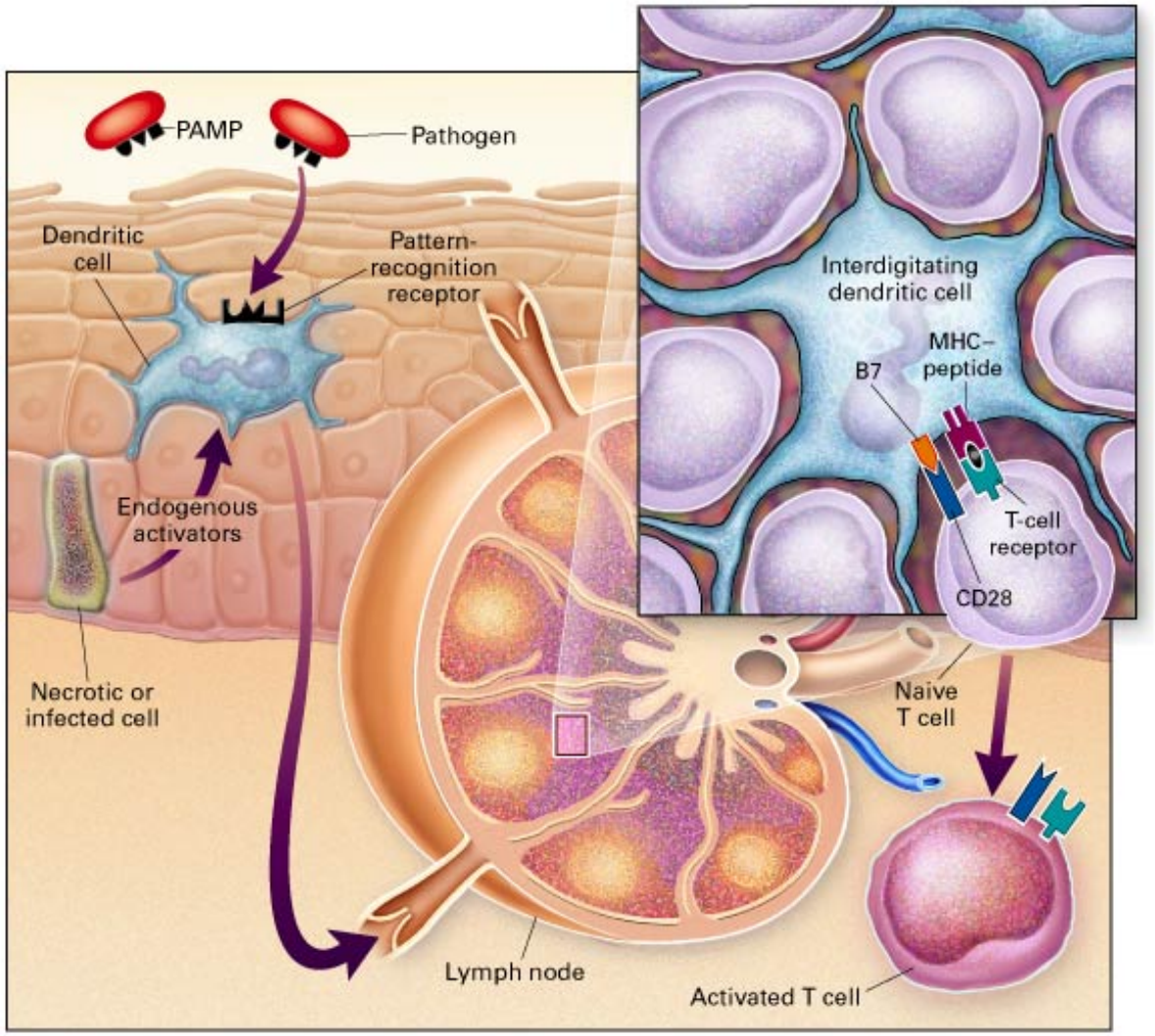


Control

Anti gD Ab

IFNs alpha/gamma

Role of DCs


PAMP
Pathogen
Dendritic cell
Pattern-recognition receptor
Endogenous activators
Necrotic or infected cell
Lymph node
Interdigitating dendritic cell
B7
MHC–peptide
T-cell receptor
CD28
Naive T cell
Activated T cell

Characteristics of split and subunit protein/peptide antigens

Eg Split – influenza, Subunit – Pertussis, HBV, HPV
Highly focused, specific response
Reduced immunogenicity and potential for escape mutants
Non-infectious, Low reactogenicity, acceptable tolerability
No or limited availability of innate defensive triggers
Adjuvants needed to compensate for lower immunogenicity
Synthetic production may be possible, facilitating supply