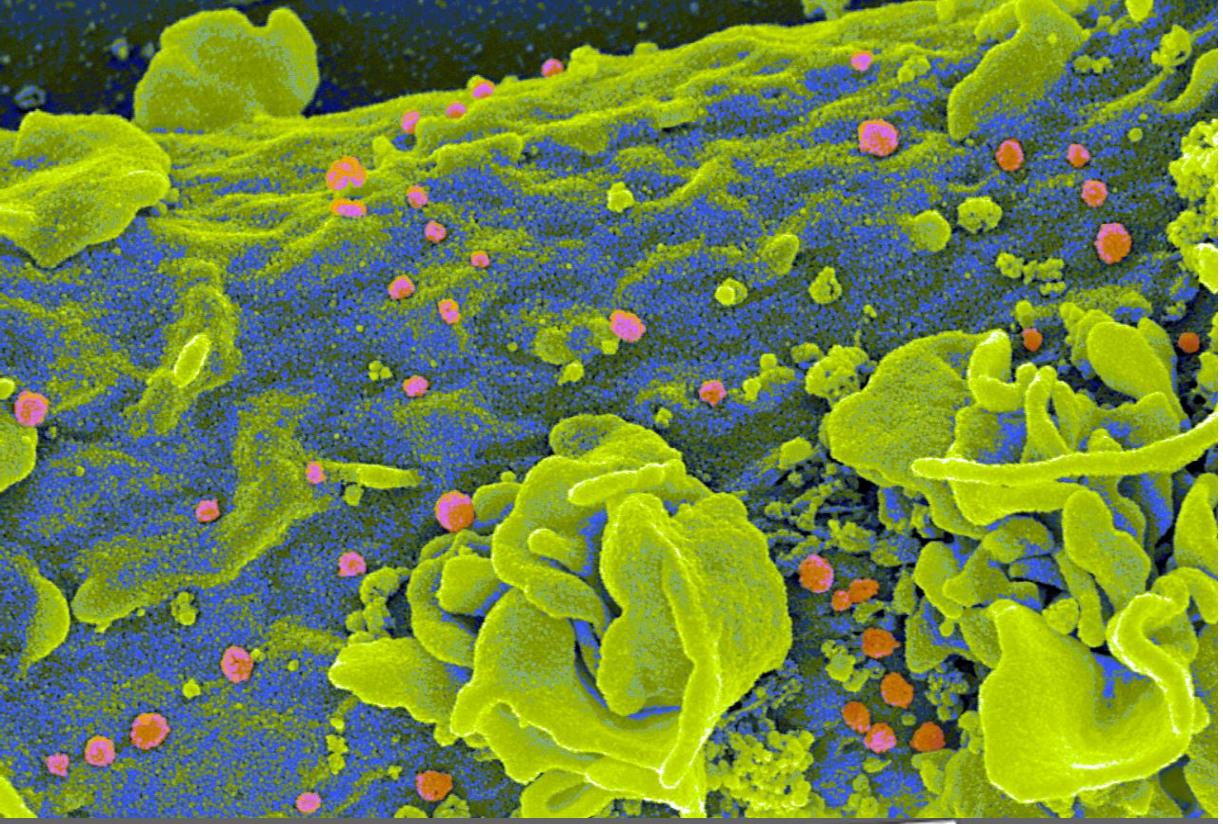
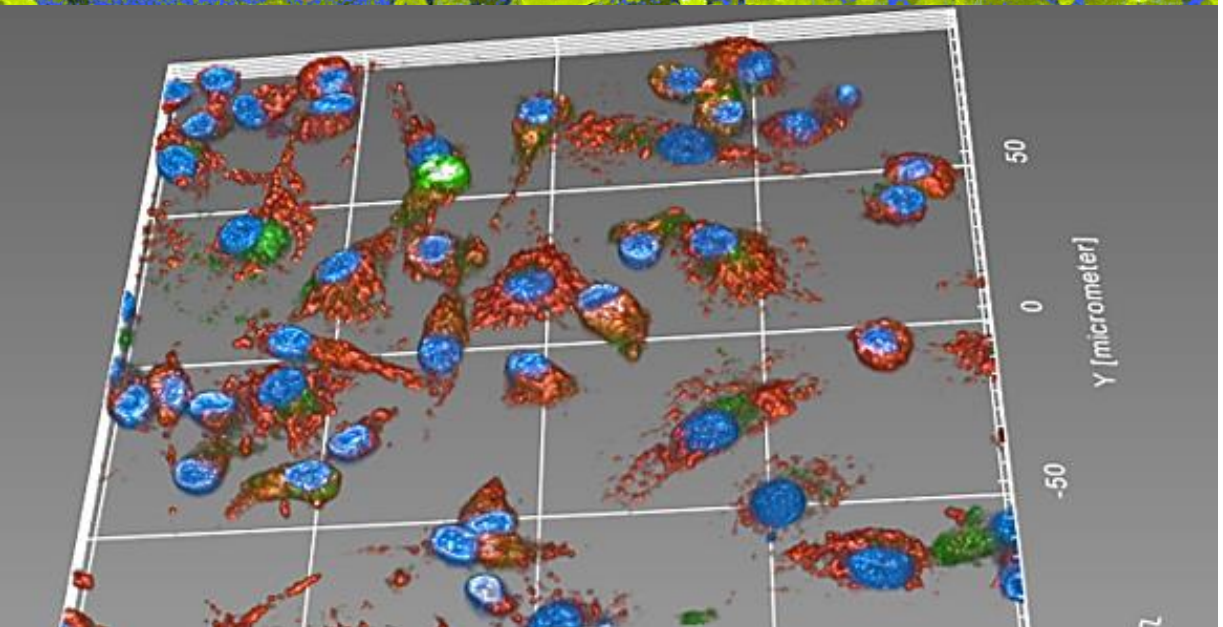
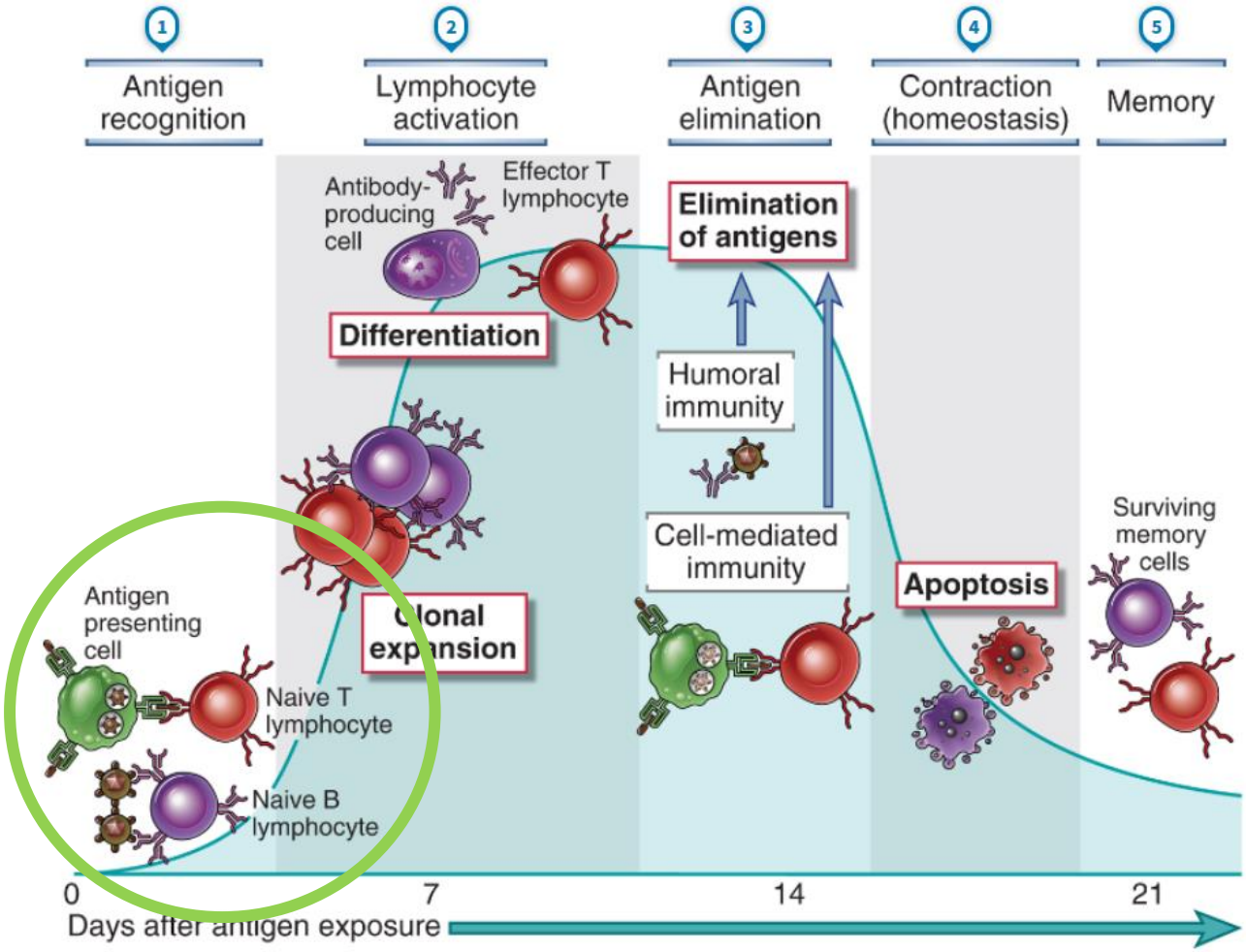




DENDRITIC CELLS

Doris Wilflingseder
Assoc.-Prof., Vice Director
Division of Hygiene and Medical Microbiology
Medical University of Innsbruck

STEPS DURING AG EXPOSURE



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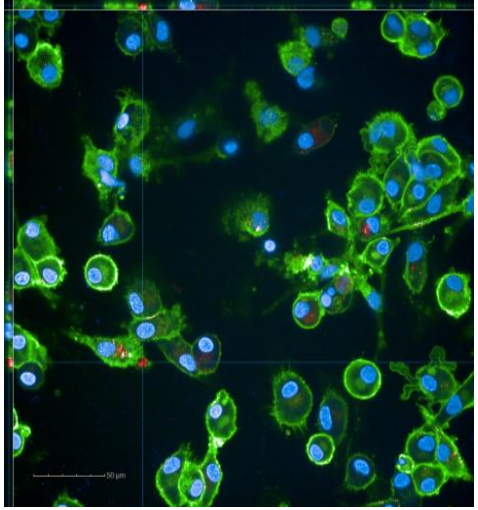
Dendritic cells (DCs)



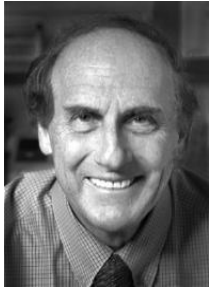
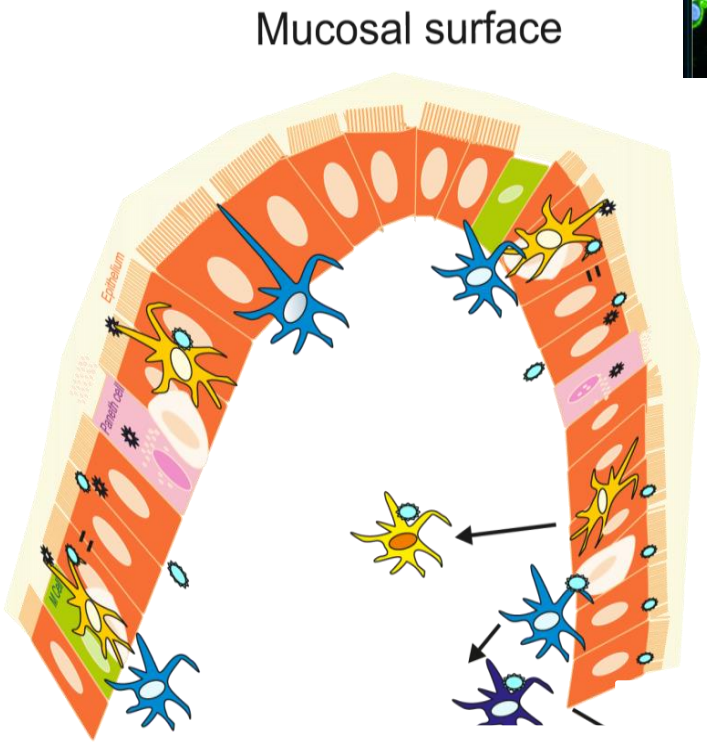


Foto: The Rockefeller University

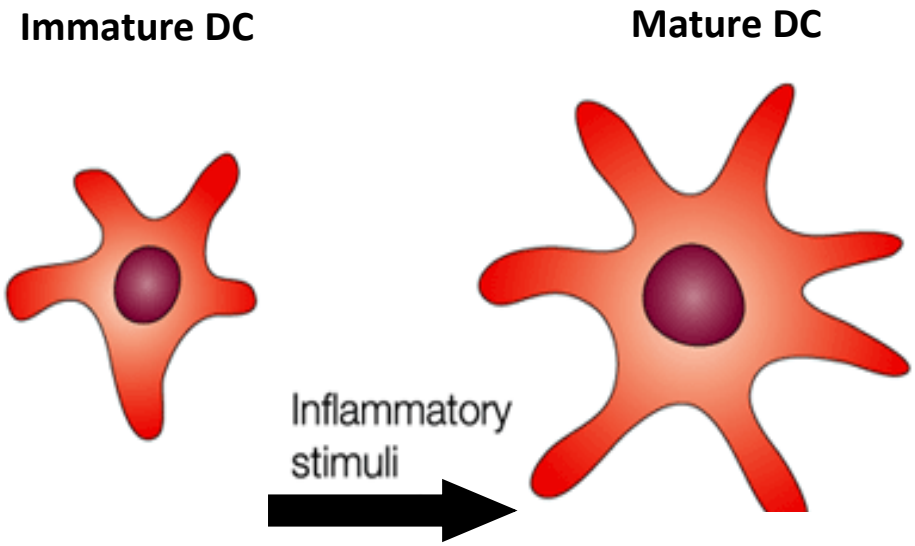
Steinmann & Cohn 1973 – Nobel Prize 2011

Sentinel function

AG capture, processing and maturation



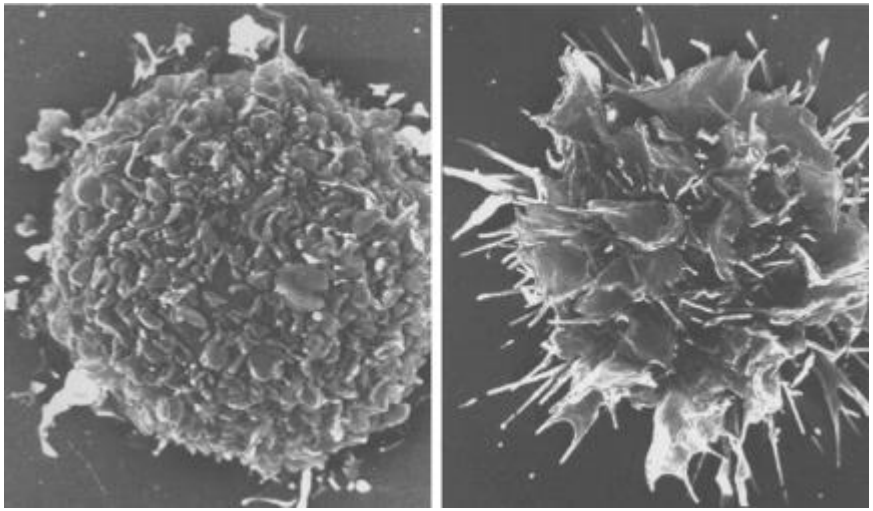
DENDRITIC CELLS – BRIDGE BETWEEN INNATE AND ADAPTIVE IMMUNITY



CD80/CD86	low		high
MHC class II	low		high
CD83	no		high
C-type lectins (eg.DC-SIGN)	high		low
Phagocytosis	high		low
CCR7	low	CCL19(MIP-3β), CCL21(SLC) → Migration to LTs	high

DC MATURATION

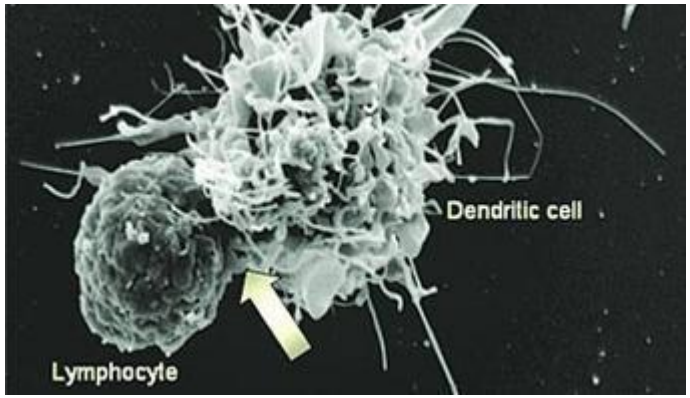
immature DC (iDC) - Periphery



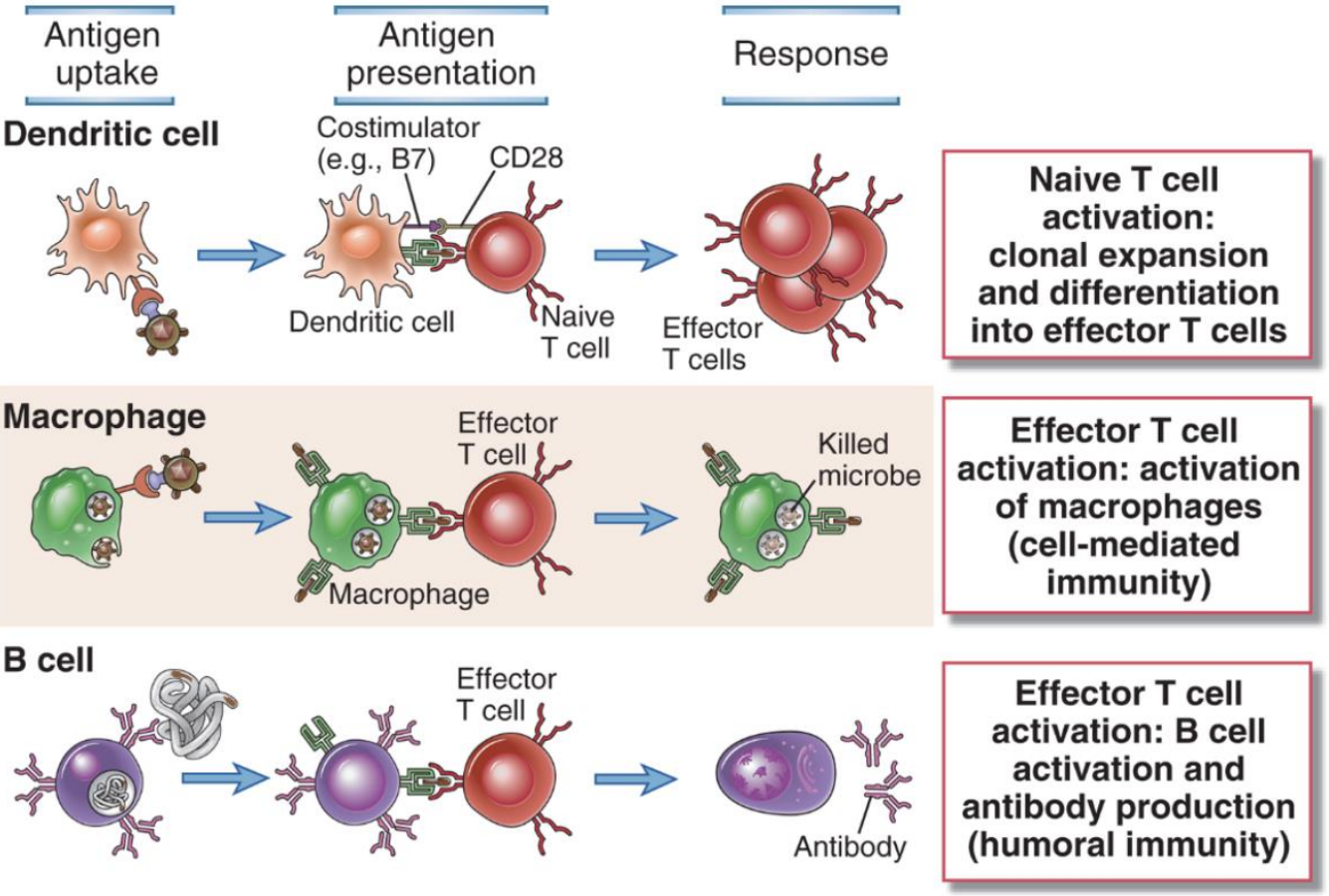
mature DC (mDC) – Lymph node

Bild: Nikolaus Romani

AGP within the LN – DC and T Lymphocyte



PROFESSIONAL AGP CELLS



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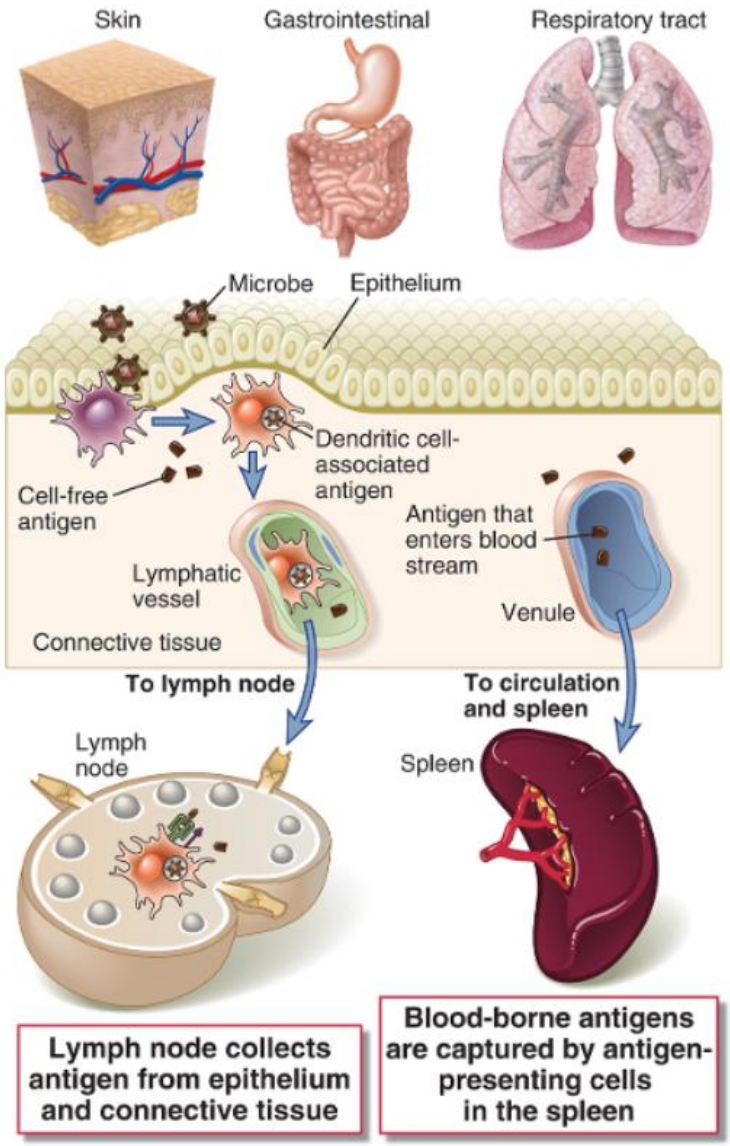
DC LOCALIZATION



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Conventional DCs

Plasmacytoid DCs

DC SUBPOPULATIONS

TABLE 6–3 The Major Subpopulations of Dendritic Cells

Feature	Conventional (Myeloid) Dendritic Cells	Plasmacytoid Dendritic Cells
Surface markers	CD11c high CD11b high	CD11c low CD11b negative B220 high
Growth factors for in vitro derivation	GM-CSF, Flt3-ligand	Flt3-ligand
Expression of Toll-like receptors (TLRs)	TLRs 4, 5, 8 high	TLRs 7, 9 high
Major cytokines produced	TNF, IL-6	Type I interferons
Postulated major functions	Induction of T cell responses against most antigens	Innate immunity and induction of T cell responses against viruses

Other subsets of dendritic cells have been described on the basis of the expression of various surface markers (such as CD4, CD8, and CD11b) or migration from tissue sites (Langerhans-type dendritic cells from epithelia and interstitial dendritic cells from tissues). Note that all DCs express class II MHC molecules. Some authorities also refer to monocyte-derived dendritic cells, which can be generated from blood monocytes cultured with various cytokines and may develop in vivo during inflammatory reactions.

DC – ANTIGEN RECOGNITION

DENDRITIC CELLS AND OTHER INNATE IMMUNE CELLS EXPRESS PRR_S RECOGNIZING PATHOGENS

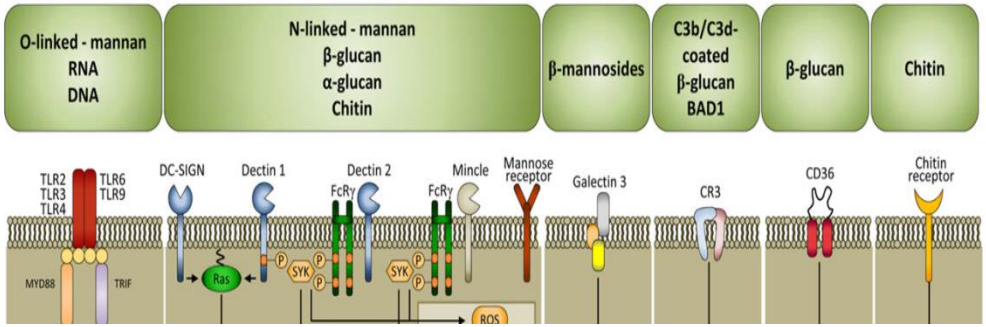
- PRRs: encoded in genome, not subject to rearrangement or variation
- PRRs: molecular sensors of infection on critical immune cells, i.e. DCs and macrophages
- but also other cells, i.e. epithelial cells, coming in contact with pathogens express subsets of PRRs

How can PRRs recognize pathogens from diverse families with diversive biology and patterns of infection in the absence of functional re-arrangement?

PRR$_S$

How can PRRs recognize pathogens from diverse families with diverse biology and patterns of infection in the absence of functional re-arrangement?

1)

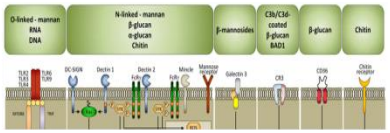


Innate IS recognizes **PAMP**s (pathogen-associated molecular patterns) > components common to many pathogens

PRR$_S$

How can PRRs recognize pathogens from diverse families with diverse biology and patterns of infection in the absence of functional re-arrangement?

1)



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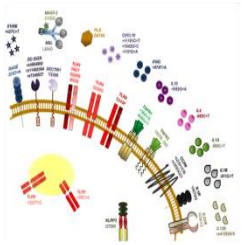

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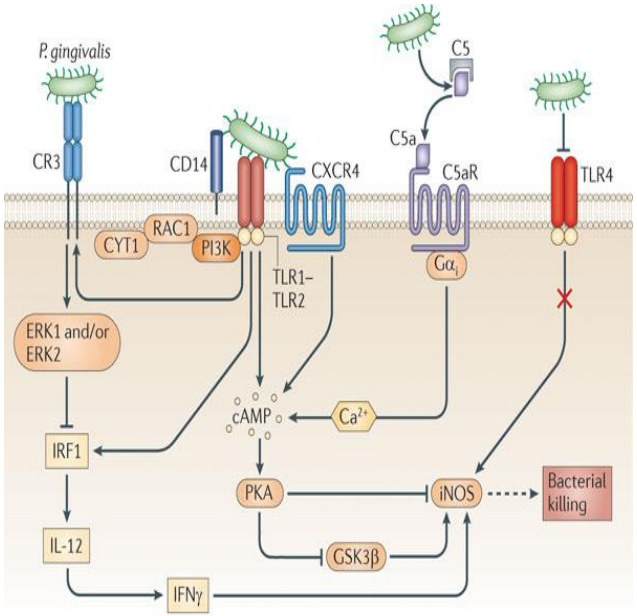
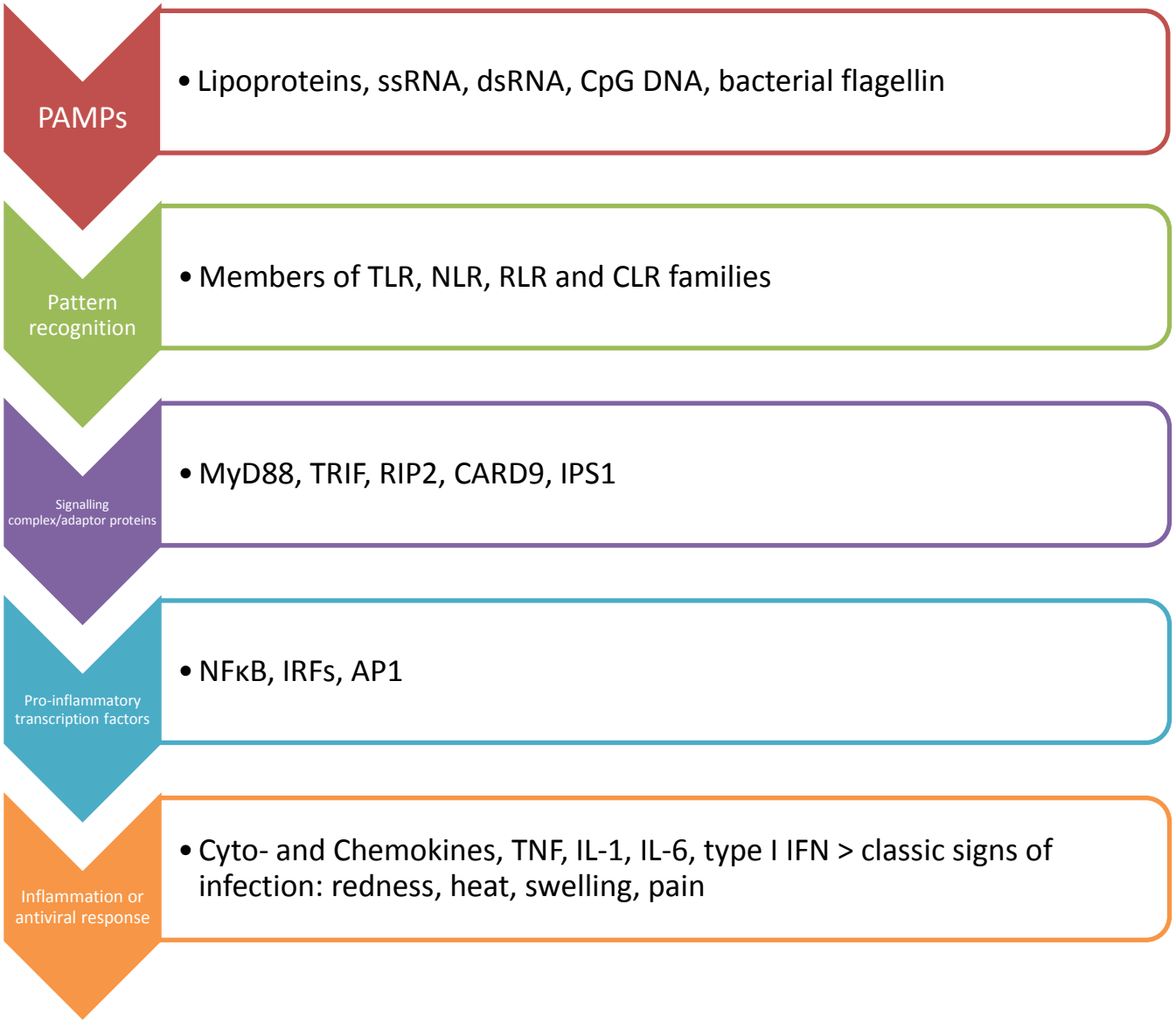


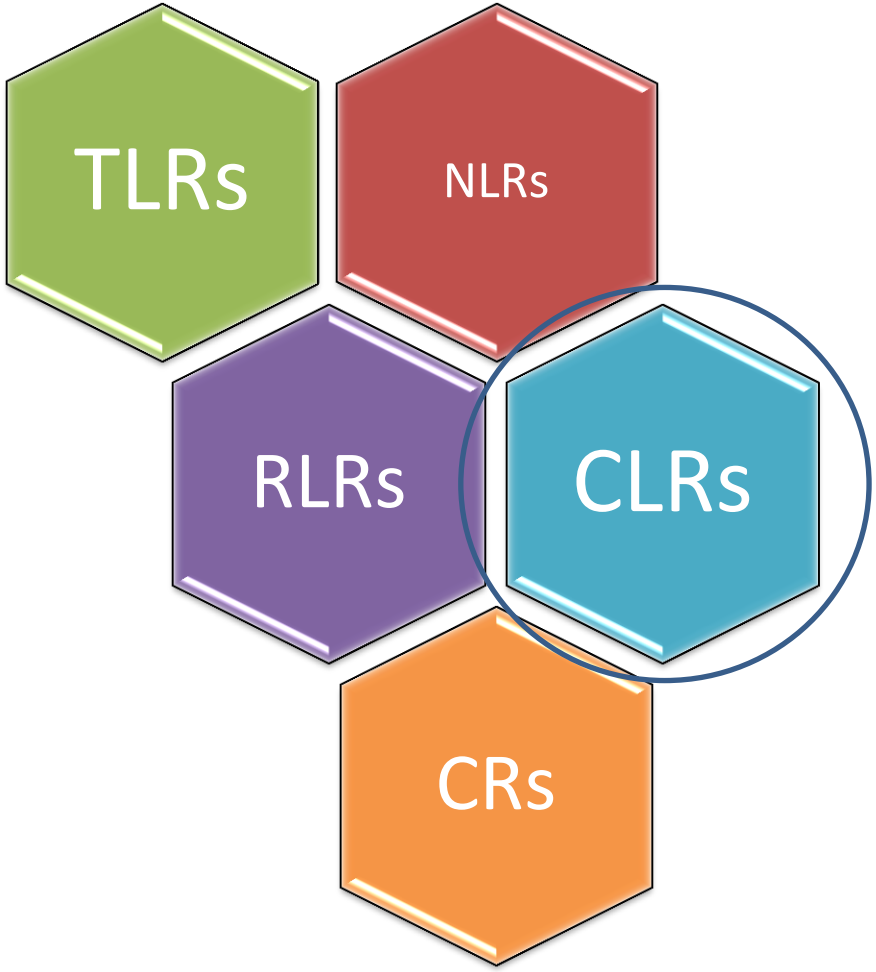
Nature Reviews | Immunology

Extensive ***receptor cross-talk*** and ***communication*** between the ***signaling*** pathways > <u>coordinated</u> response to pathogen infection

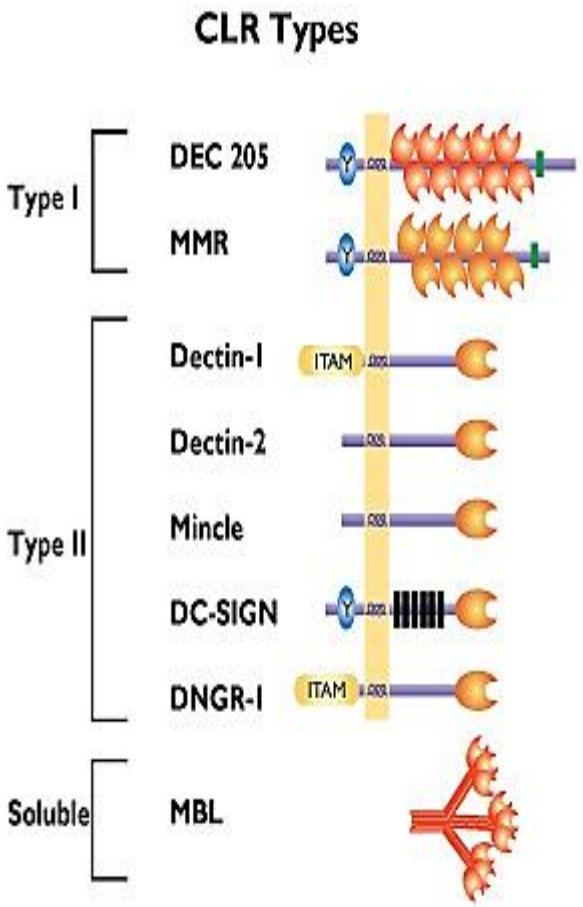
PRR$_S$ > OVERVIEW OF INNATE SIGNALING AND COMPONENTS



PRR FAMILIES



CLRs – C-type lectin receptors



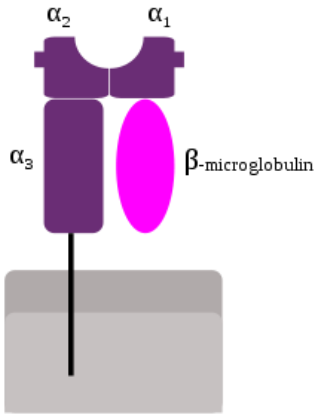
- comprise large family of PRRs
- bind to **carbohydrates** in a calcium-dependent manner
- based on molecular structures > **3 types of CLRs**: **Type I** (DEC-205, MMR), **Type II** (dectins, mincle, DC-SIGN), **soluble** (MBL)
- involved in **fungal recognition** and **modulation** of the innate immune response
- expressed by most cell types including **macrophages** and dendritic cells (**DCs**) > internalize glycoproteins and microbes to clear and present Ag to T lymphocytes

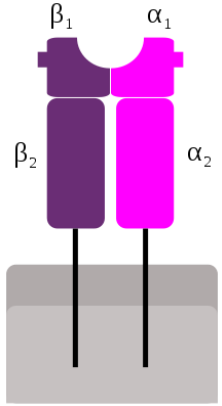
http://www.invivogen.com/review-clr

DC - ANTIGENPRESENTATION

- AGP via MHC molecules
  - 2 types: MHC-I and MHC-II
  - MHC-I expressed on all nucleated cells
  - MHC-I present self and non-self Ags on the surface
  - **MHC-II only expressed on professional APCs, e.g. DCs**

MHC-I molecule





MHC-II molecule

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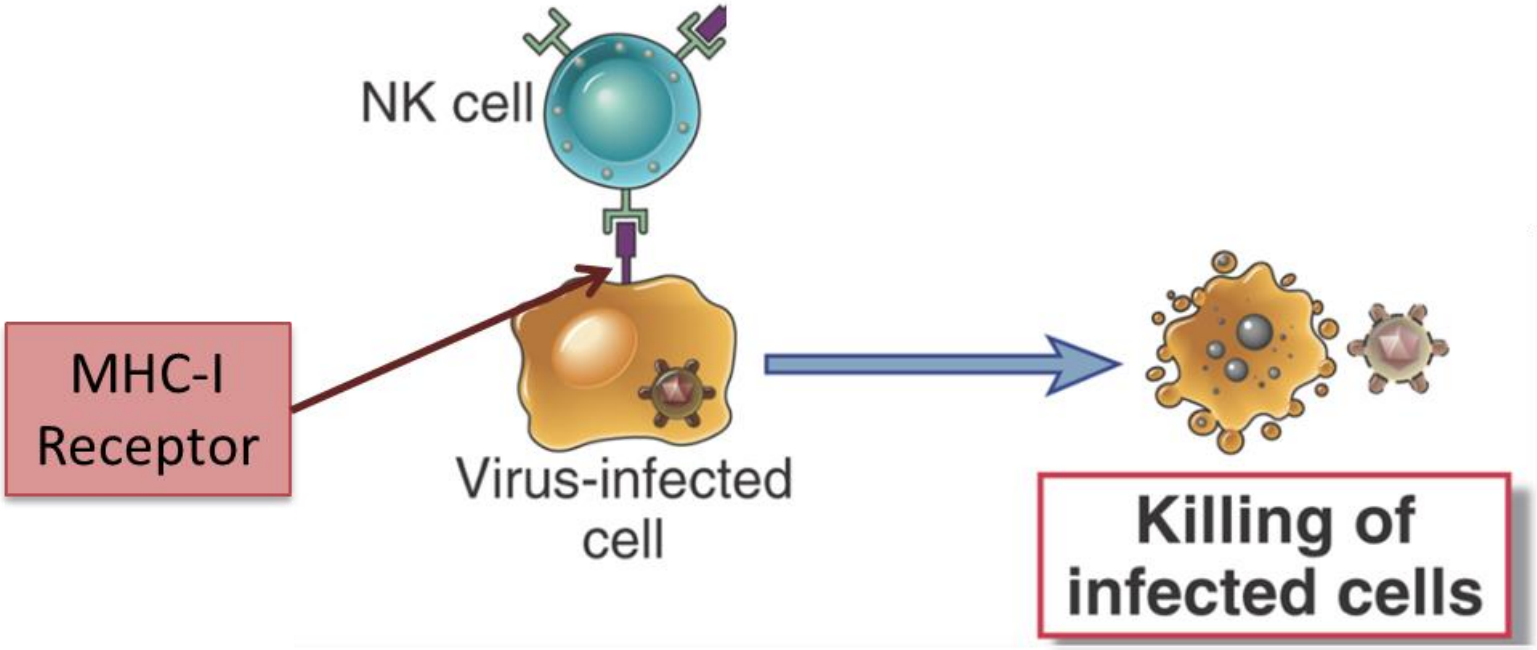
ANTIGENPRESENTATION – MHC I

- MHC-I facilitate ´VIEW inside cells´
- **Self** and **non-self antigens** from inside cells are ligated to MHC-I and are presented on the surface to e.g. NK cells or $CD8^+$ T cells



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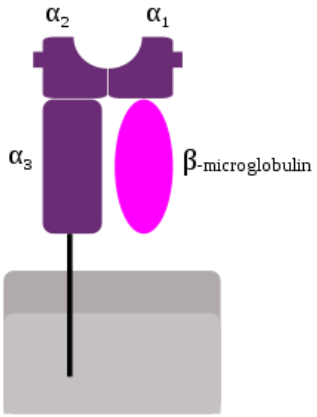
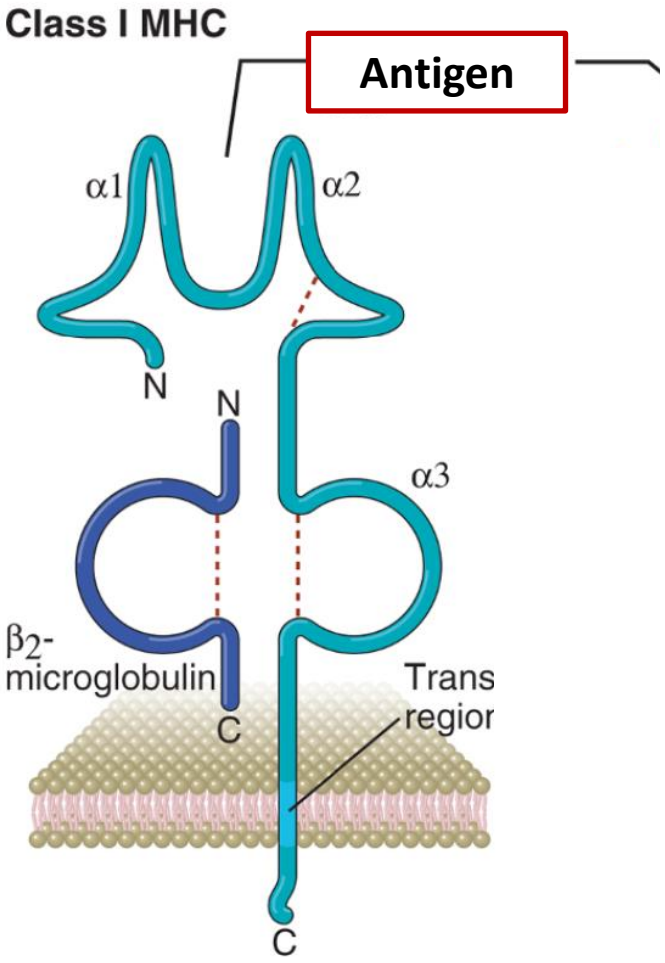
ANTIGENPRESENTATION – MHC I

MHC-I





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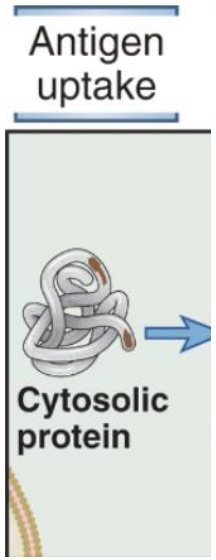
ANTIGENPRESENTATION – MHC I

- Course of pathogen recognition via MHC I
  - Pathogens (cytosolic proteins) INSIDE cells
  - Pathogens processed by proteosome – Ags formed
  - Ags transported to MHC-I via TAP



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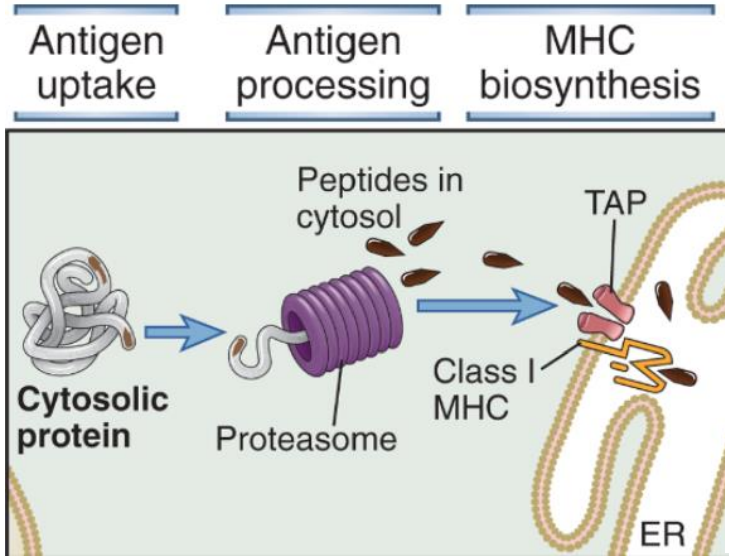
ANTIGENPRESENTATION – MHC I

- Course of pathogen recognition via MHC I
  - Ags loaded onto MHC-I (peptide-MHC association)
  - Ag-MHC-I complexes transported to the cell surface by vesicles



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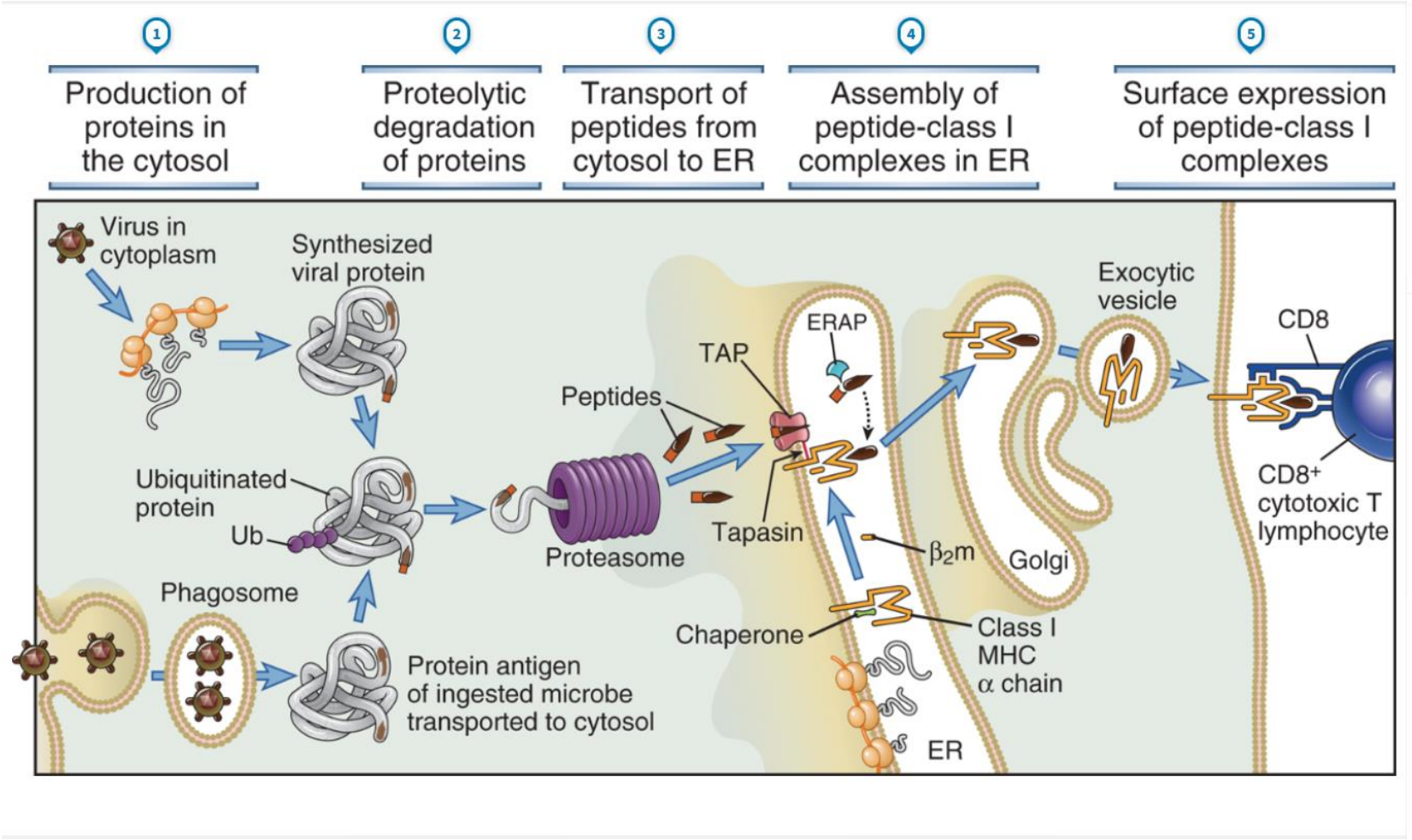
ANTIGENPRESENTATION – MHC I



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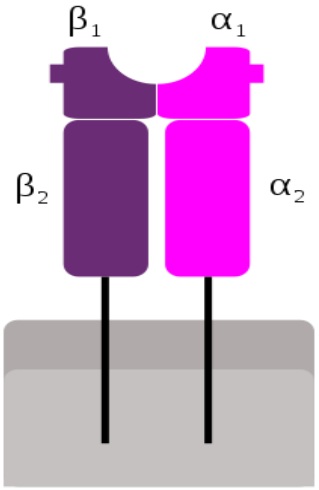
ANTIGENPRESENTATION – MHC II

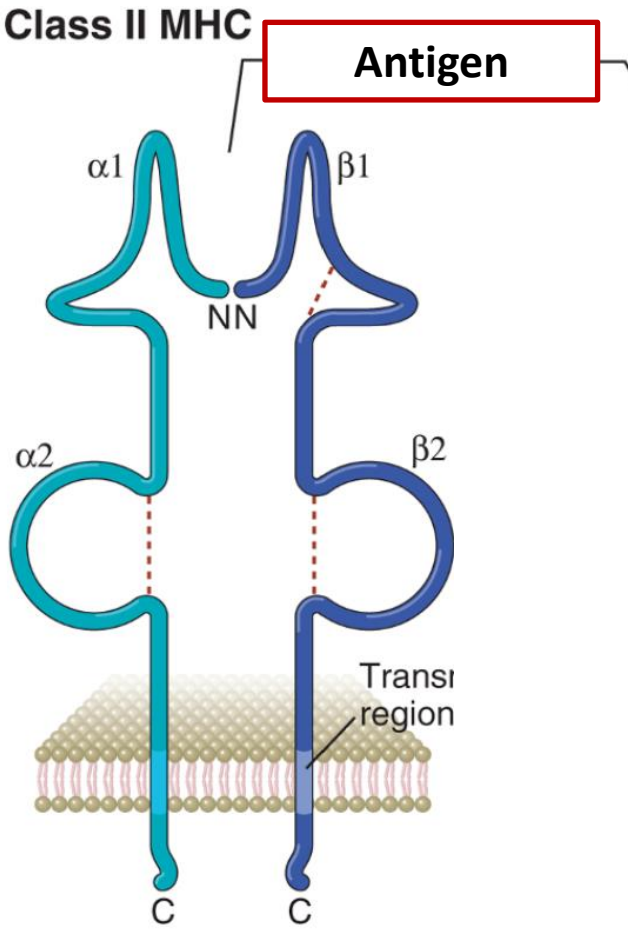
- Surface of professional AGP cells
- Also presentation of proteins, but not from INSIDE!
- Pathogens are recognized by AGP cells and internalized
- AGP cells process pathogens in small fragments > Ags
- Once stimulated by up-take DCs migrate to the proximate lymph node and present Ags to naive T cells >
  Induction of adaptive immunity

Peripheral pathogens are recognized via MHC II and an adaptive immune response is activated within lymphatic tissues

ANTIGENPRESENTATION – MHC II



MHC-II molecule



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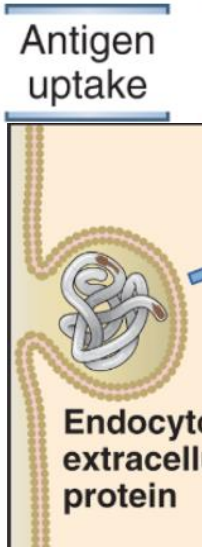
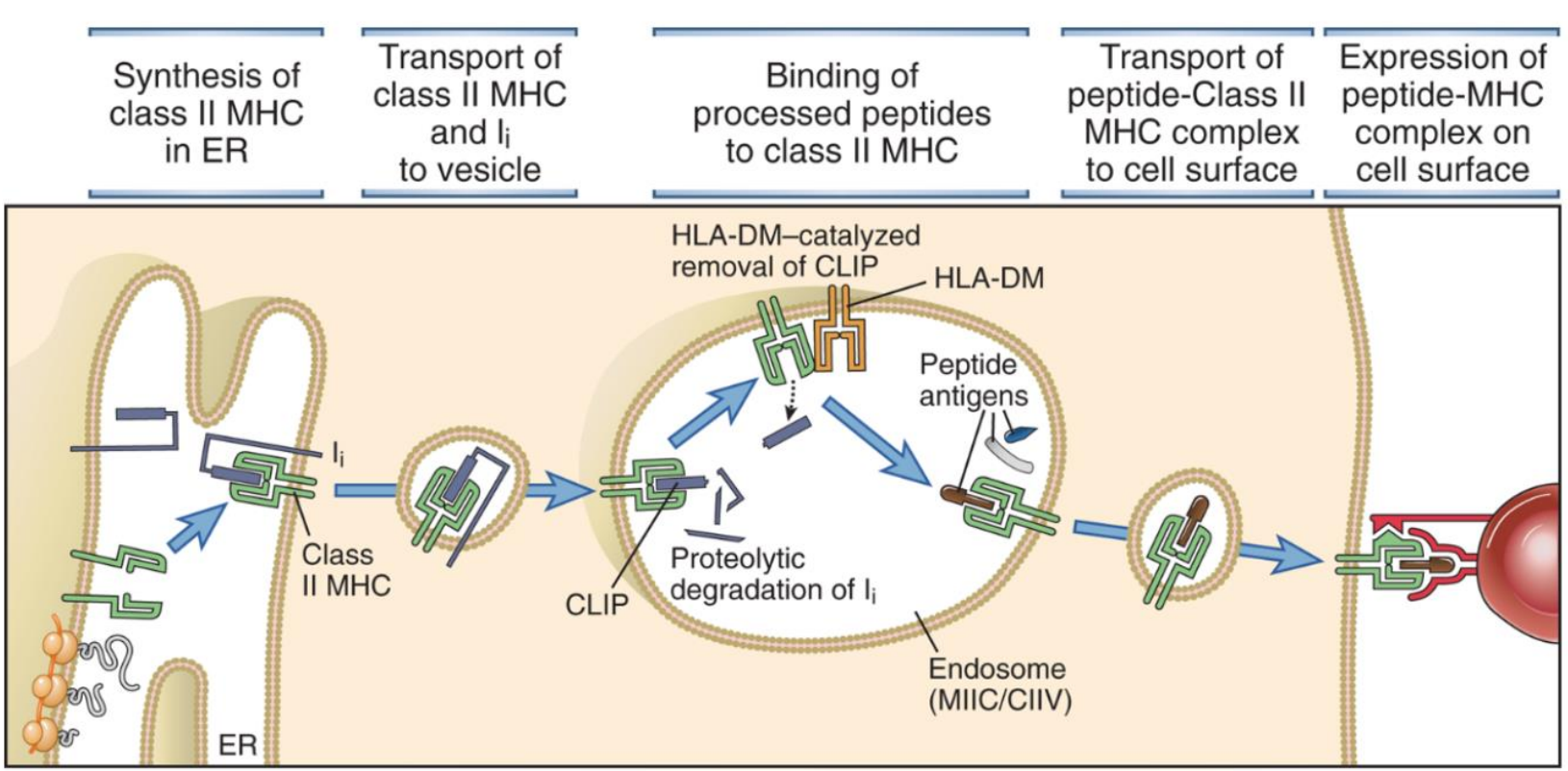
ANTIGENPRESENTATION – MHC II

- **Course of MHC II AGP**

  - Extracellular pathogens processed via MHC II
  - Up-take of pathogens by phagocytosis
  - Ag packing into vesicles and fusion with MHC class II vesicles
  - Presentation at DC surface



ANTIGENPRESENTATION – MHC II



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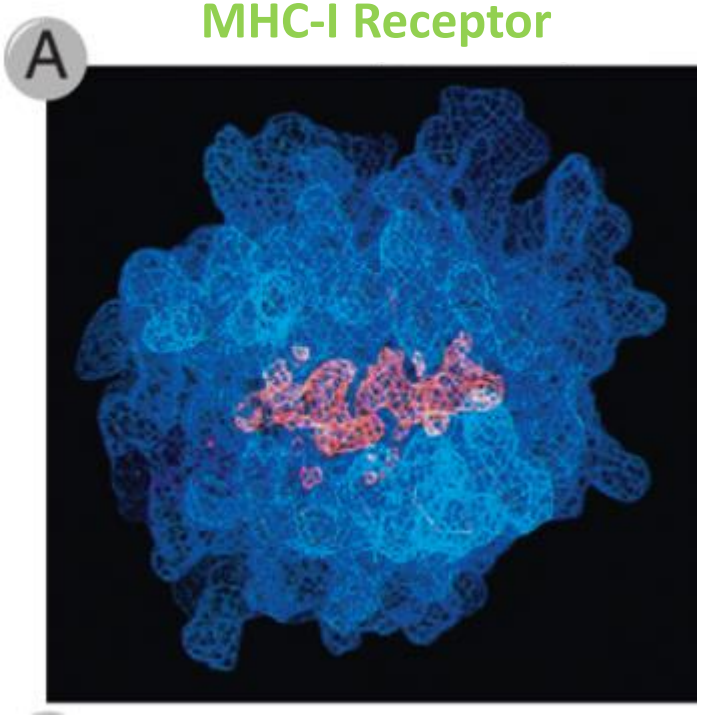
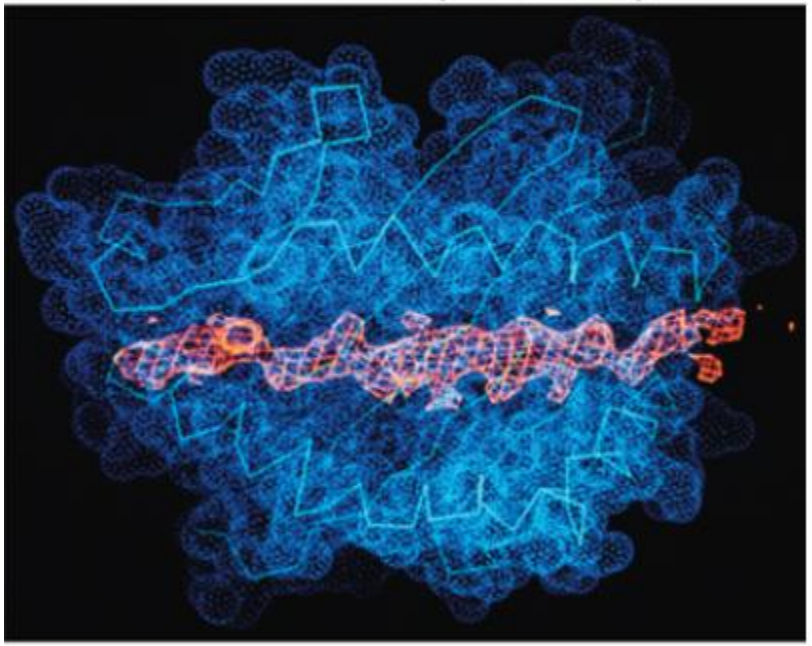
ANTIGENPRESENTATION – Comparison MHC I/MHC II

MHC-I Receptor



MHC-II Receptor



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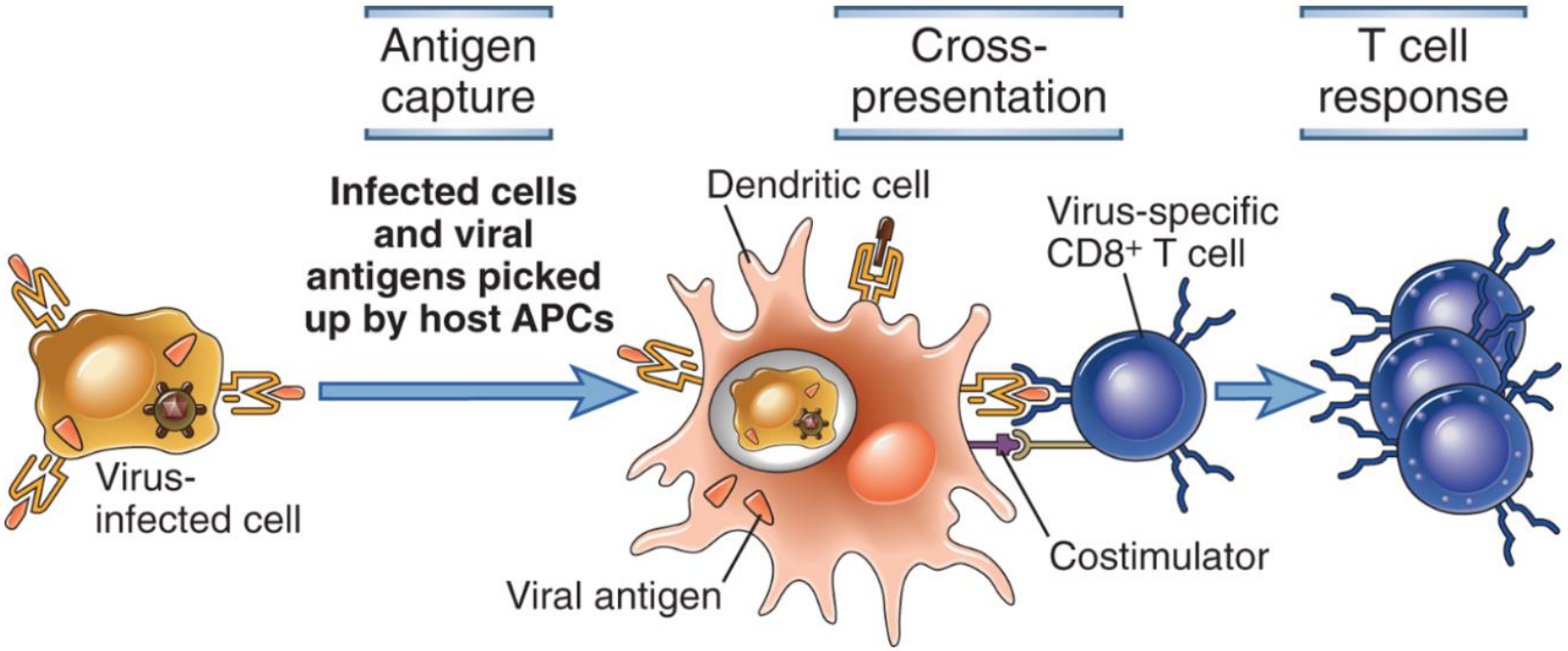
ANTIGENPRESENTATION – Cross-presentation



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INNATE IMMUNITY TO VIRUSES

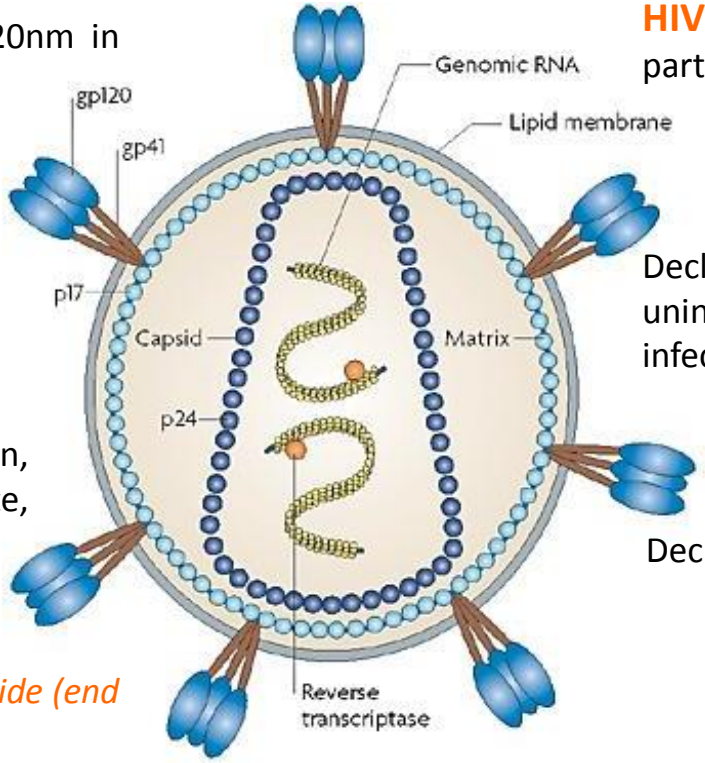
RECOGNITION OF / INNATE IMMUNE RESPONSE TO HIV-1

HIV-1 – positive ssRNA virus, 120nm in diameter

acquired immunodeficiency syndrome (*AIDS*) > progressive failure of IS (opportunistic infections, cancer)

Transfer with bodily fluids (semen, blood, vaginal fluid, pre-ejaculate, breast-milk)

36.700.000 *people infected worldwide (end of 2015)*



HIV-1 – infects vital cells in IS (in particular $CD4^+$ T cells, macrophages, DCs)

↓

Decline in **$CD4^+$ T cells** (apoptosis of uninfected by-standers, direct killing of infected cells)

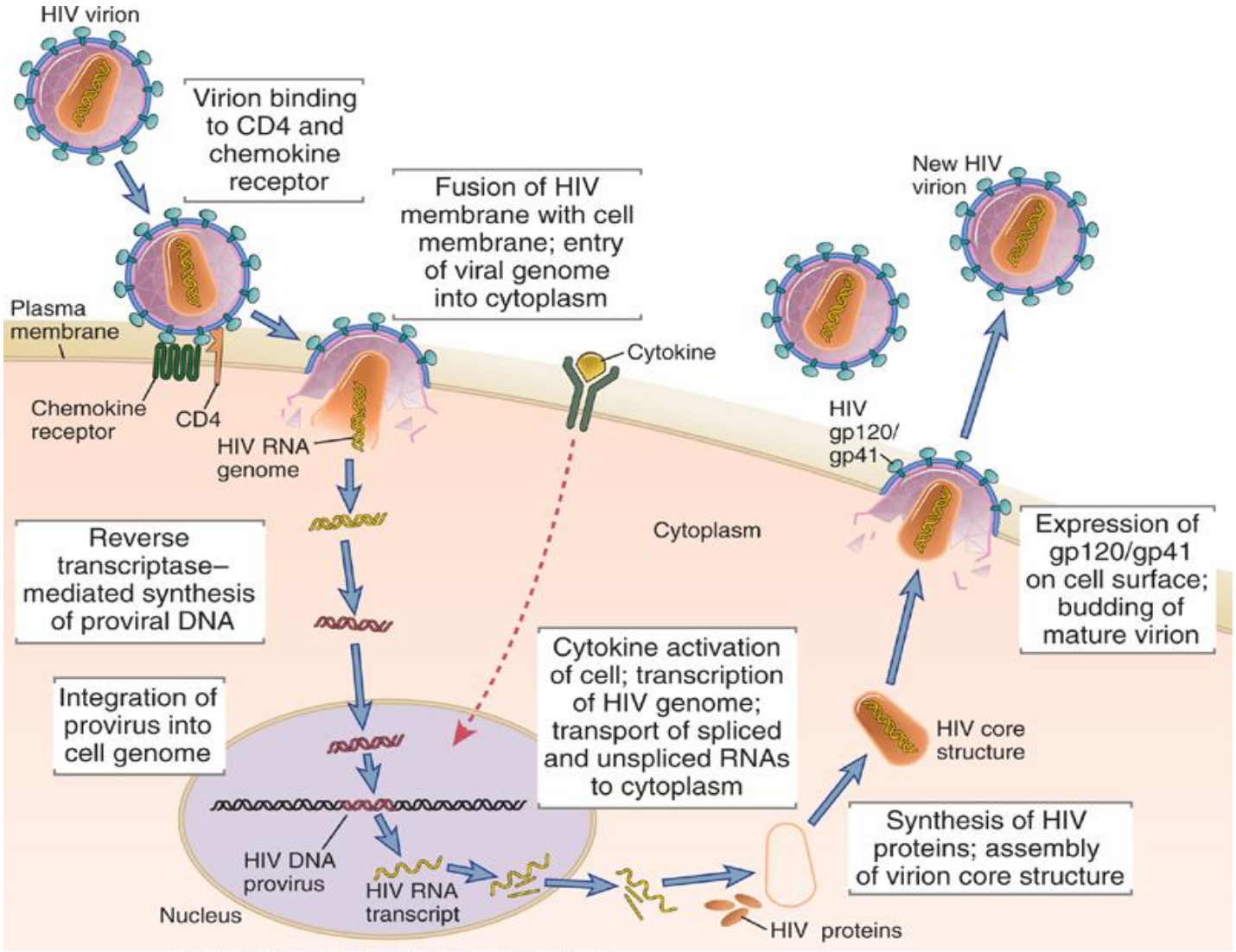
↓

Decline in $CD4^+$ T cells below a critical level > *Loss of cell-mediated immunity*

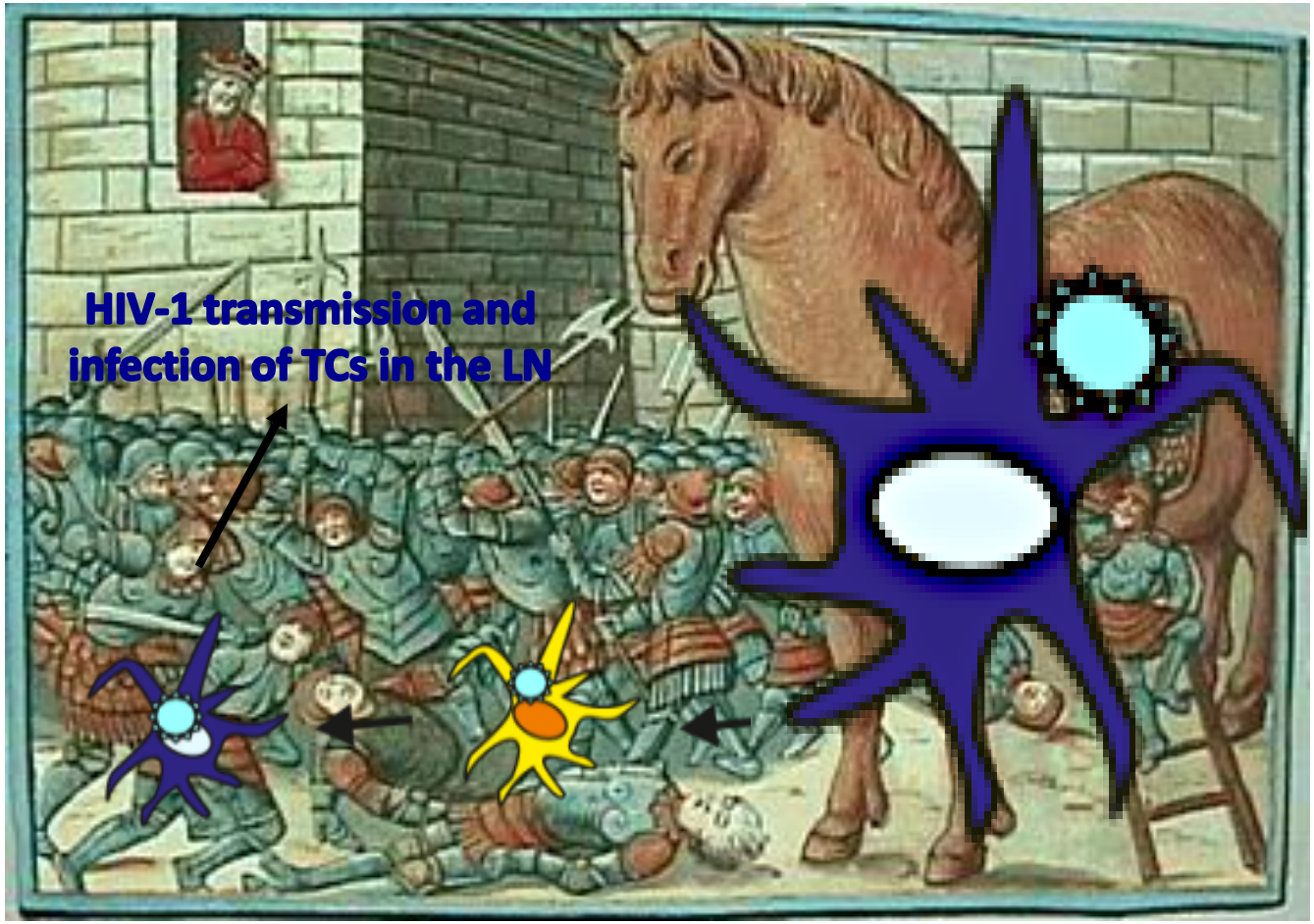
↓

Body becomes progressively more susceptible to opportunistic infections/cancers

HIV-1 LIFE CYCLE

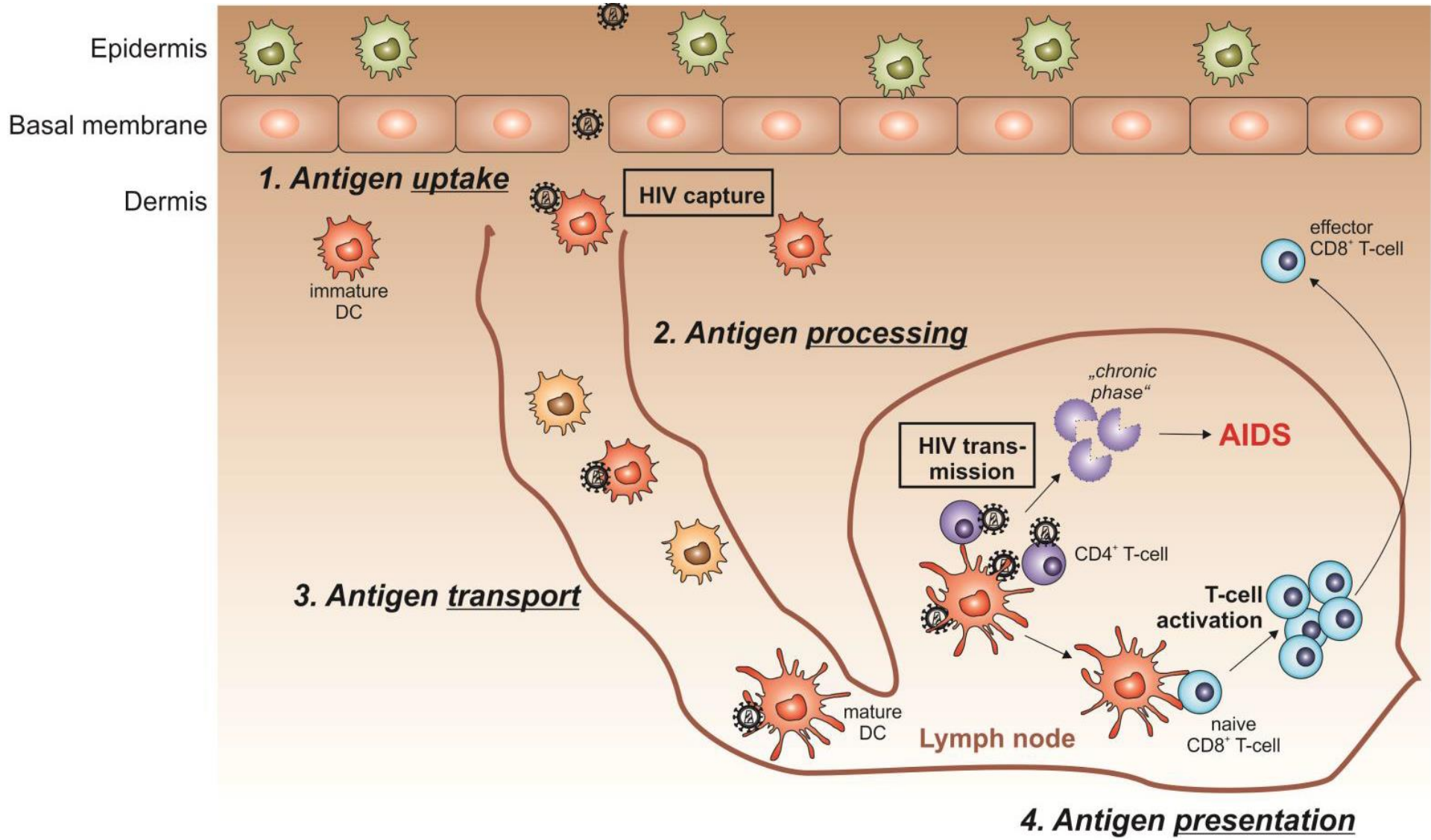


WHY/HOW is HIV-1 able to evade efficient AGP from DCs and develop chronic disease?

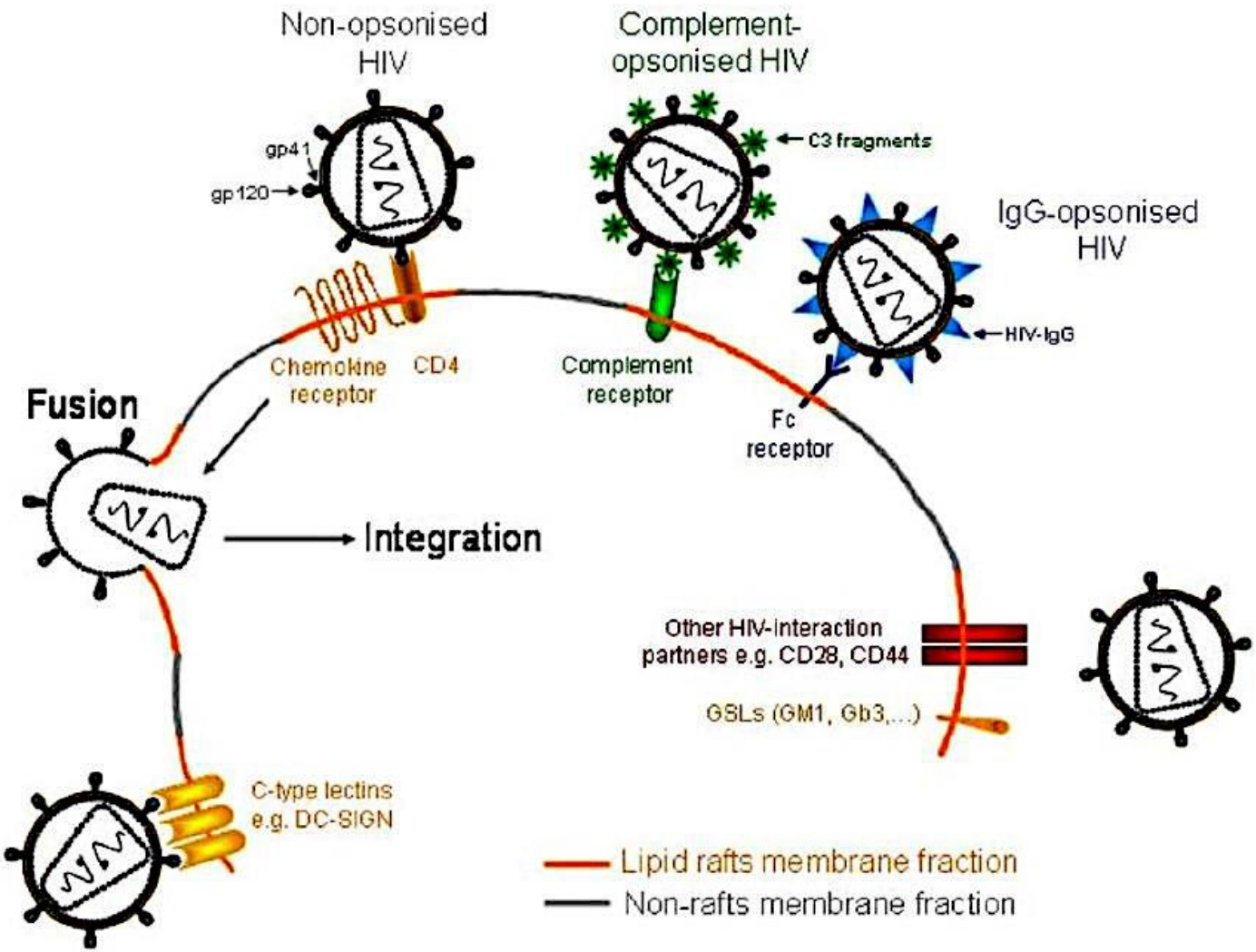


TROJAN HORSE HYPOTHESIS

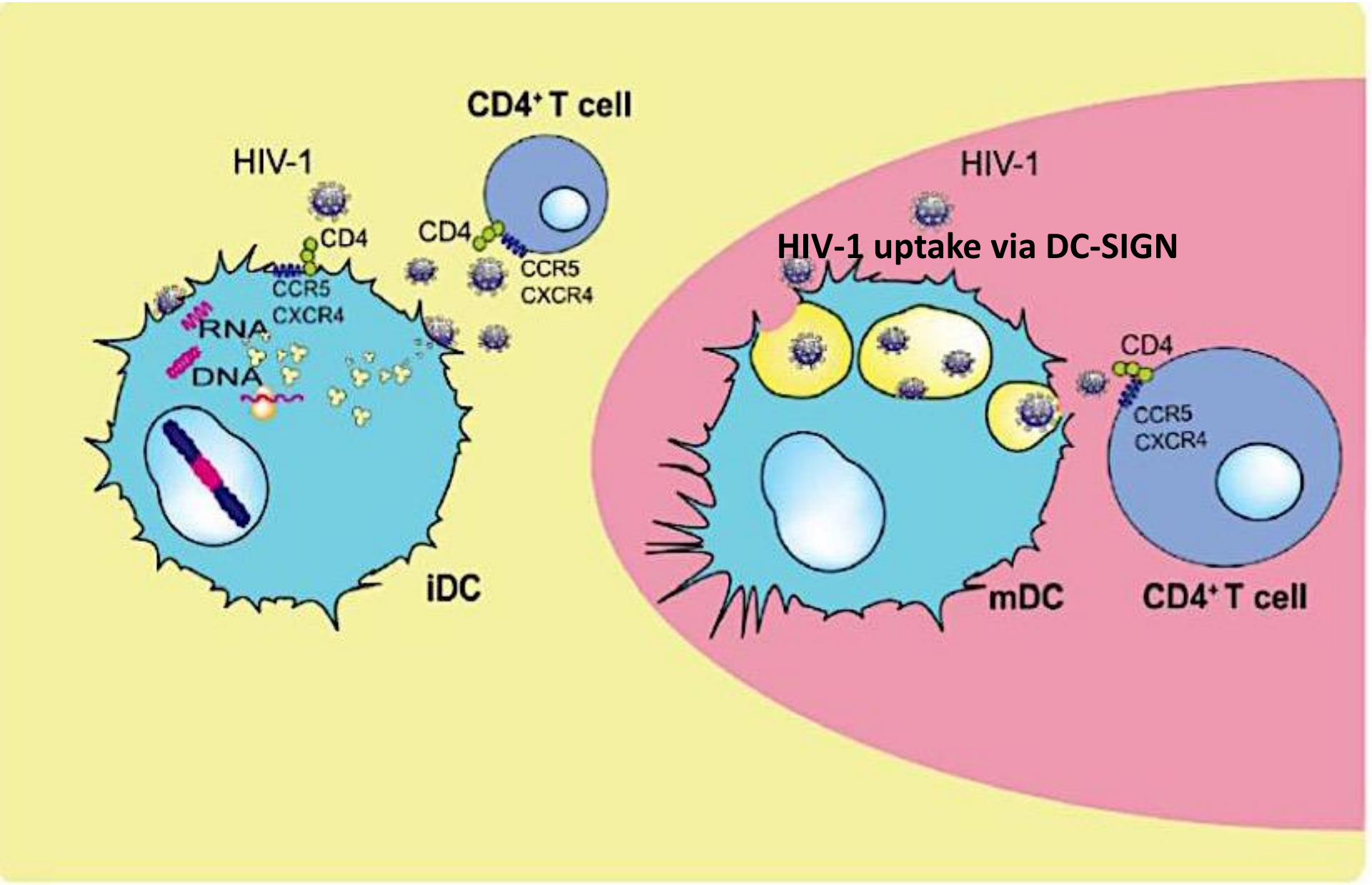
Role of DCs in HIV-1 pathogenesis



Initial attachment of HIV-1 to host DCs



HIV-1 Transfer from iDCs/mDCs



Izquierdo-Useros *et al*. [2010]

nature immunology

HIV-1 blocks the signaling adaptor MAVS to evade antiviral host defense after sensing of abortive HIV-1 RNA by the host helicase DDX3

Sonja I Gringhuis, Nina Hertoghs, Tanja M Kaptein, Esther M Zijlstra-Willems, Ramin Sarrami-Forooshani, Joris K Sprokholt, Nienke H van Teijlingen, Neeltje A Kootstra, Thijs Booiman, Karel A van Dort, Carla M S Ribeiro, Agata Drewniak & Teunis B H Geijtenbeek

The mechanisms by which human immunodeficiency virus 1 (HIV-1) avoids immune surveillance by dendritic cells (DCs), and thereby prevents protective adaptive immune responses, remain poorly understood. Here we showed that HIV-1 actively arrested antiviral immune responses by DCs, which contributed to efficient HIV-1 replication in infected individuals. We identified the RNA helicase DDX3 as an HIV-1 sensor that bound abortive HIV-1 RNA after HIV-1 infection and induced DC maturation and type I interferon responses via the signaling adaptor MAVS. Notably, **HIV-1 recognition by the C-type lectin receptor DC-SIGN activated the mitotic kinase PLK1, which suppressed signaling downstream of MAVS, thereby interfering with intrinsic host defense during HIV-1 infection**. Finally, we showed that PLK1-mediated suppression of DDX3–MAVS signaling was a viral strategy that accelerated HIV-1 replication in infected individuals.

nature immunology

HIV-1 blocks the signaling adaptor MAVS to evade antiviral host defense after sensing of abortive HIV-1 RNA by the host helicase DDX3

- DCs do not mount protective immunity against HIV-1
  - Normally after PRR recognition, **type I IFNs** are induced, which activate an antiviral program of interferon-stimulated genes (ISGs) > ISGs counteract virus replication.
  - Type I interferons also induce antiviral adaptive immunity **via DC maturation** and T helper cell polarization

  - HIV-1 infects DCs, BUT neither **DC activation** nor **type I interferon** responses are induced (low-level productive infection)

  - Although viral RNA, DNA and proteins are recognized by various host proteins (DC-SIGN, RIG-I, TLR8, SAMHD1, TREX1, **DDX3, MAVS** etc.), it remains unclear how HIV-1 avoids immune surveillance in DCs.

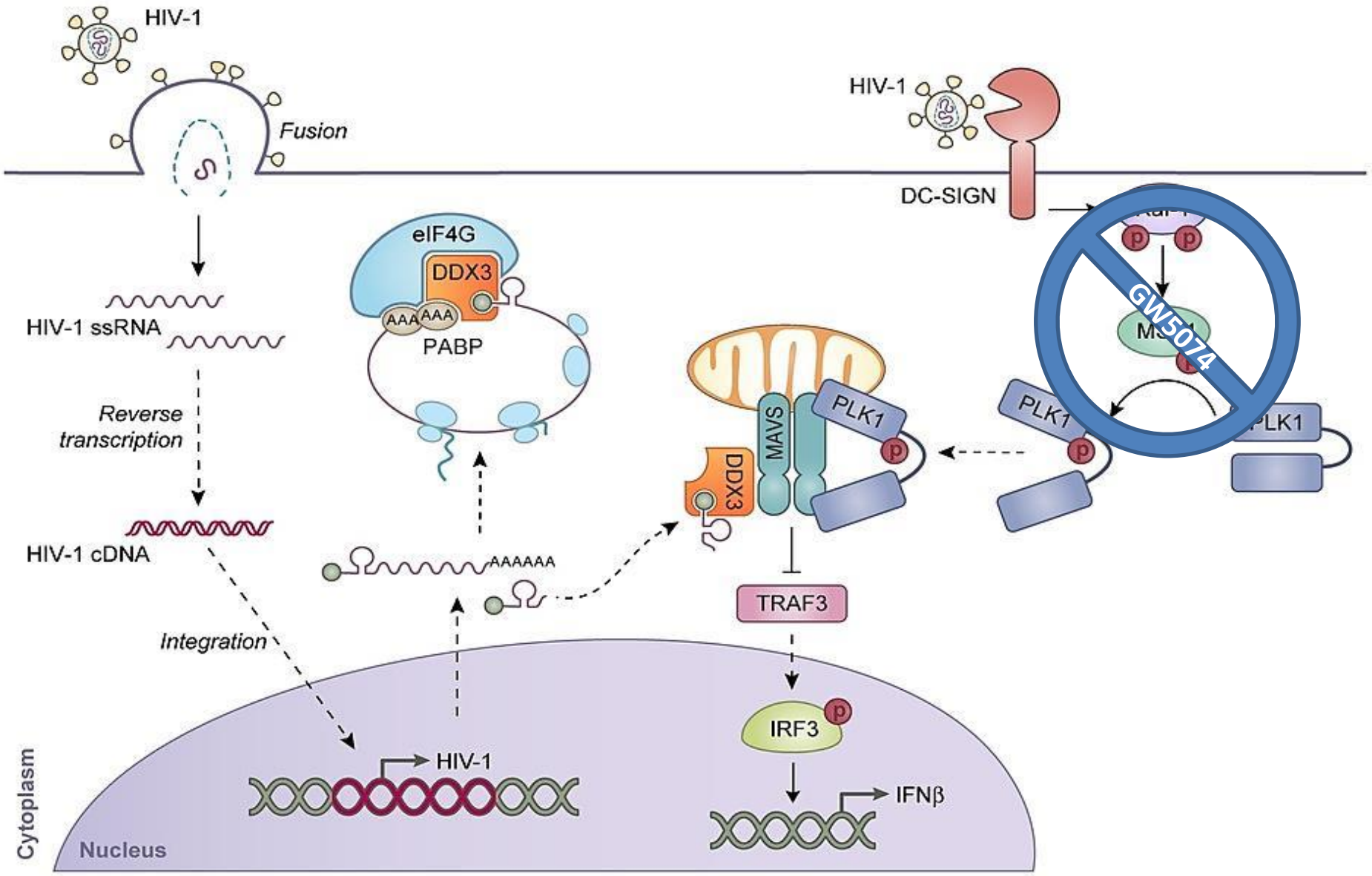
  - In this manuscript, one piece in the puzzle of inefficient DC activation and antiviral functions upon HIV-1 infection was shed light on >

    group identified RNA helicase DDX3 as sensor for abortive HIV-1 RNA in DCs, which induced IFN I and DC maturation via signaling adaptor protein MAVS.

BUT: simultaneous recognition of HIV-1 by DC-SIGN suppressed these responses via Raf-1-mediated activation of host factor PLK1, which impeded signaling downstream of MAVS.

nature immunology

HIV-1 blocks the signaling adaptor MAVS to evade antiviral host defense after sensing of abortive HIV-1 RNA by the host helicase DDX3



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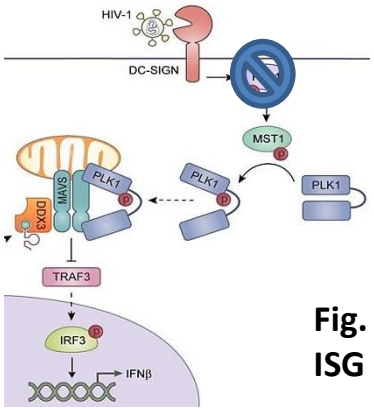
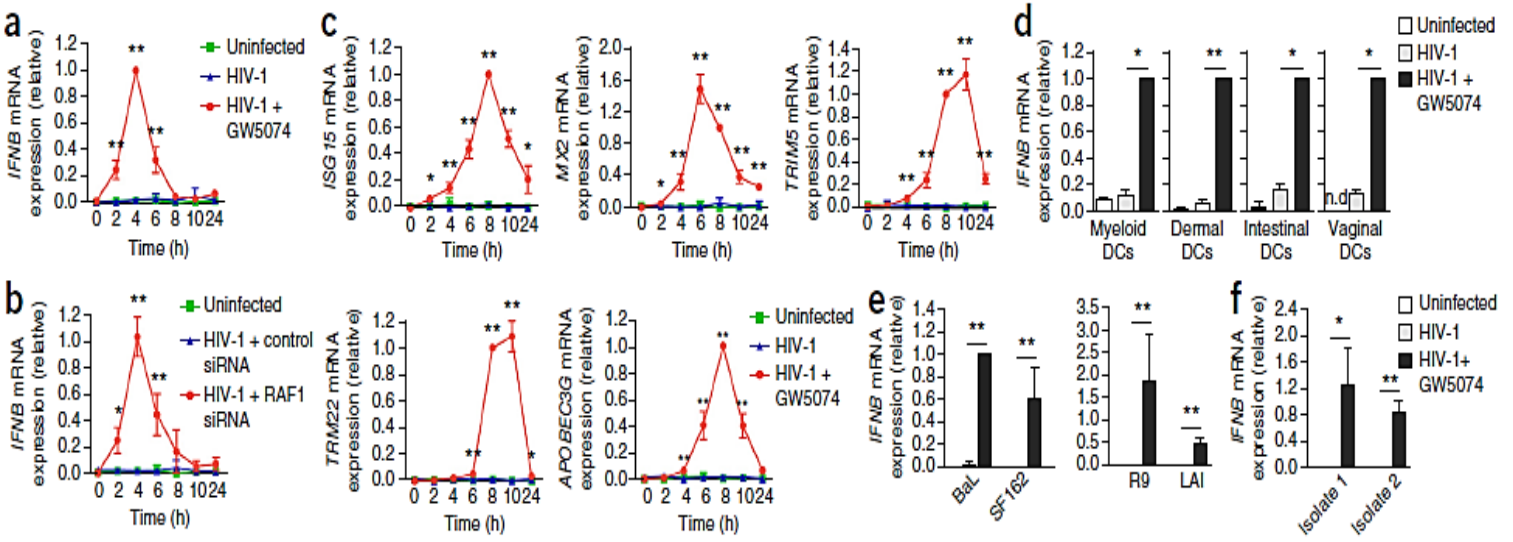


Fig. 1 Raf-1 inactivation triggers type I IFN responses in DCs (real-time RT-PCR of type I IFN and ISG responses)



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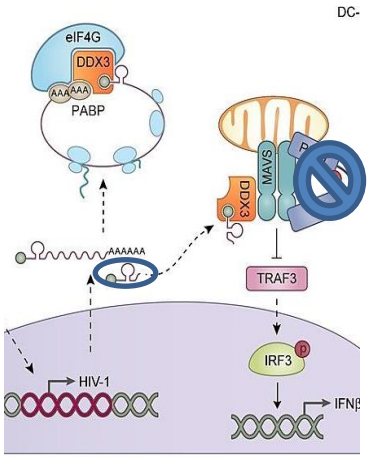

Fig. 2 Efficient type I IFN responses are mediated via DDX3 and MAVS (real-time RT-PCR using siRNAs, confocal co-localization).

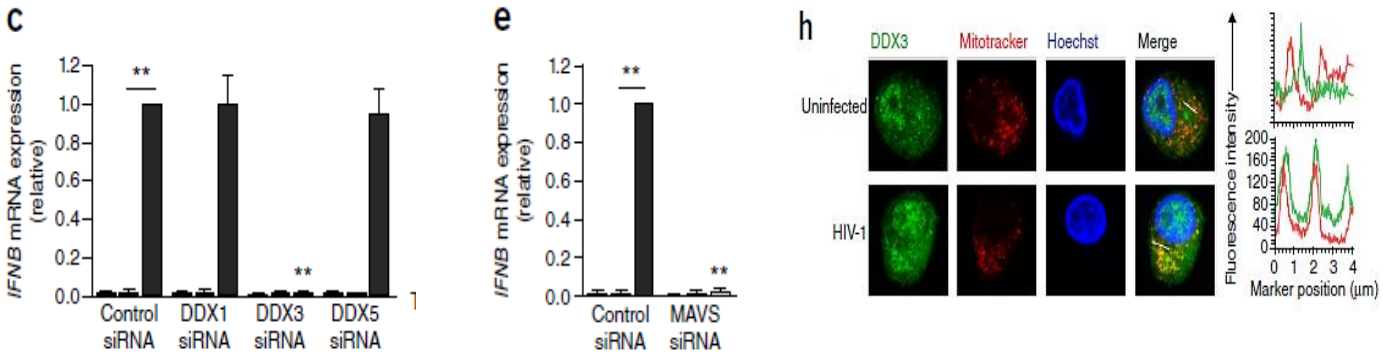

Fig. 3 DDX3 bound to capped abortive HIV-1 RNA products induces type I interferon responses via MAVS

- See, whether DDX3 associates with MAVS within translation initiation complexes (TIC) at ribosomes > IB of TIC after pulldown, RT-PCR of abortive and tat-rev mRNA after IP)

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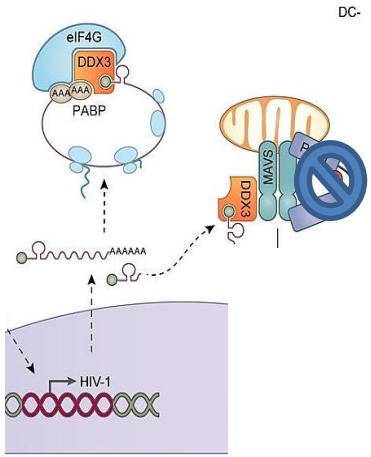

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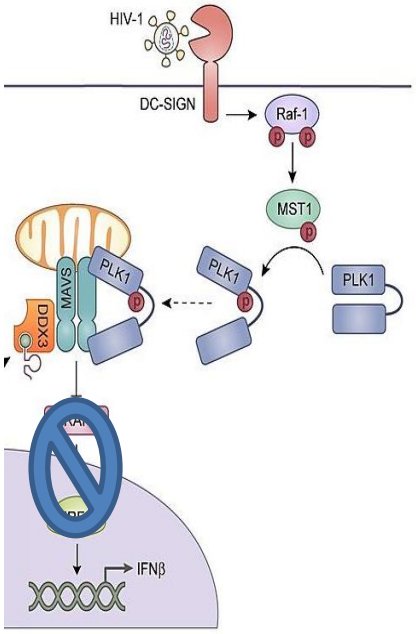
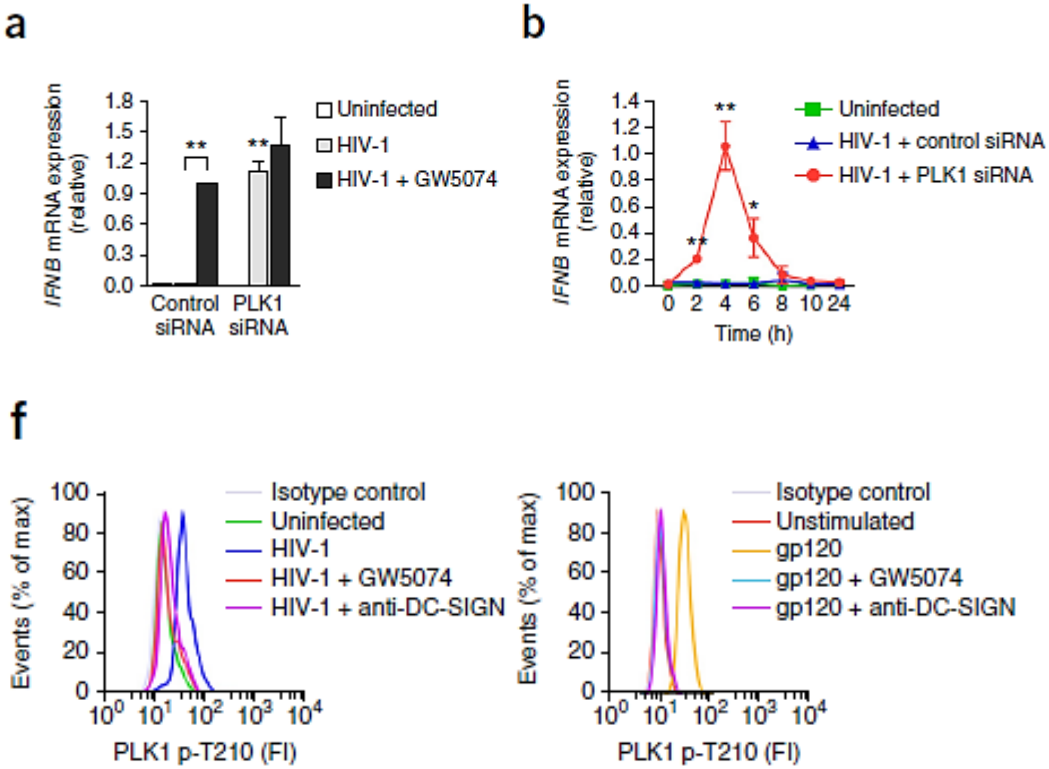


Fig. 5 HIV-1 attachment via DC-SIGN activates the Raf-1 signalosome (MST1, adapter CNK1) and PLK1. PLK1 activation in turn blocks TRAF3 recruitment to the DDX3-MAVS complex and thus also IFN I responses.



Association of PLK1 with MAVS in HIV-1-infected cells prevents downstream DDX3-MAVS signaling.

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HIV-1 blocks the signaling adaptor MAVS to evade antiviral host defense after sensing of abortive HIV-1 RNA by the host helicase DDX3

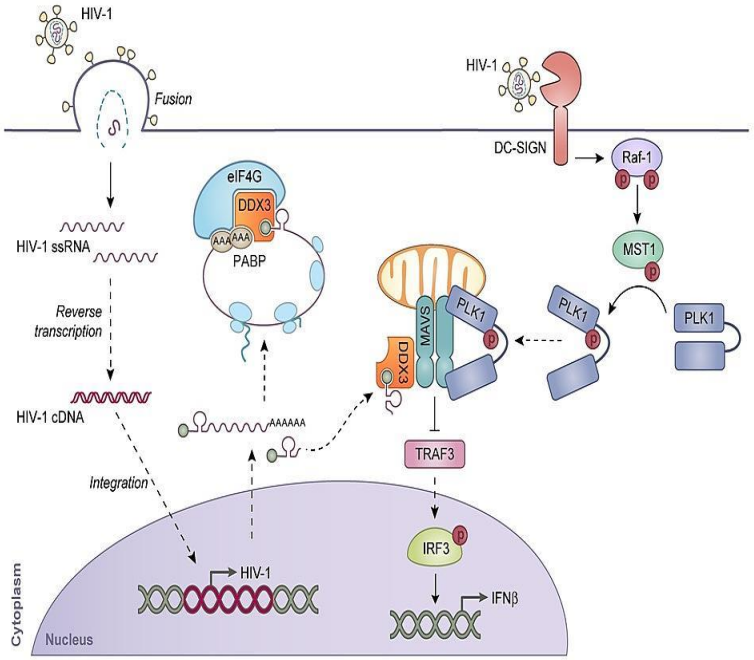
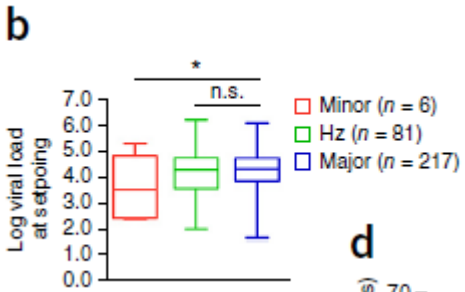
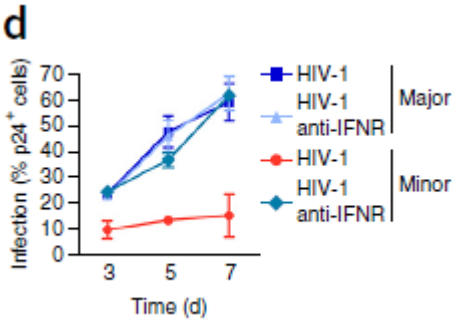


Fig. 6/7 Type I IFN responses suppress HIV-1 replication.

Effect of SNPs in genes encoding DDX3-MAVS signaling components within a cohort of untreated HIV-1-infected MSM were analyzed next. 3 SNPs w/i MAVS (rs7262903, rs7269329, rs7267297) with minor and major genotype were analyzed.

- *Minor and major genotypes* diverge from ancestor virus (probably due to evolutionary pressures by the host).
- Found that plasma viral load at set point was significantly lower in HIV-1 infected individuals homozygous for the minor alleles of rs7262903 and rs7269329 (minor genotype, rare MAVS allele) than in individuals homozygous for major alleles (major genotype) or heterozygous individuals.
- Found that moDCs from healthy donors with the minor genotype illustrated lower infection rates and higher type I IFN expression after infection with HIV-1 compared to moDCs with major or heterozygous genotype.





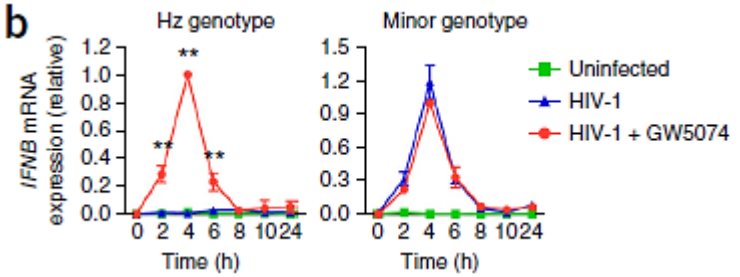


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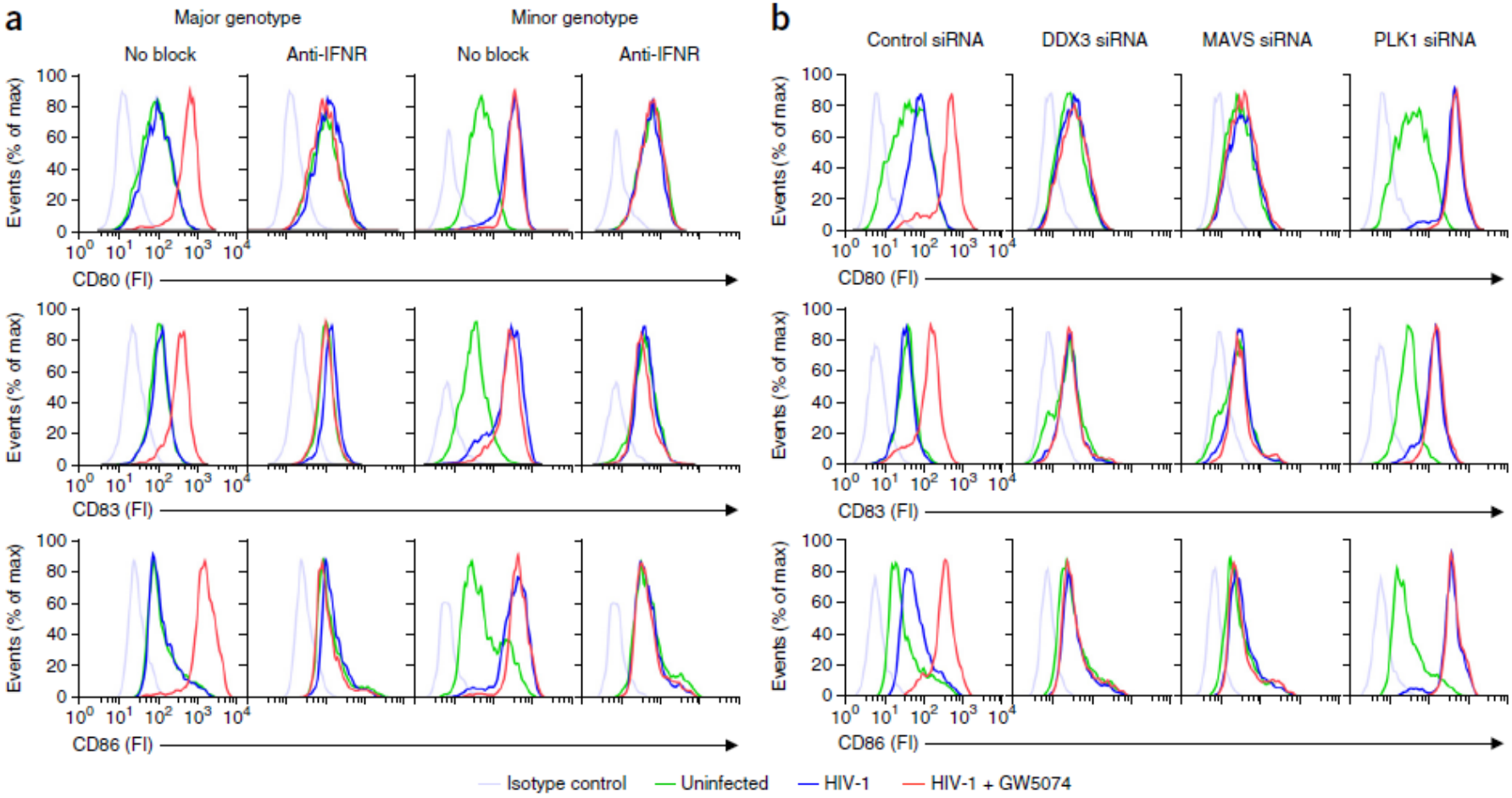
Fig. 8 DC activation in major and minor genotypes and upon siRNA silencing of DDX3, MAVS or PLK1.



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Highlights

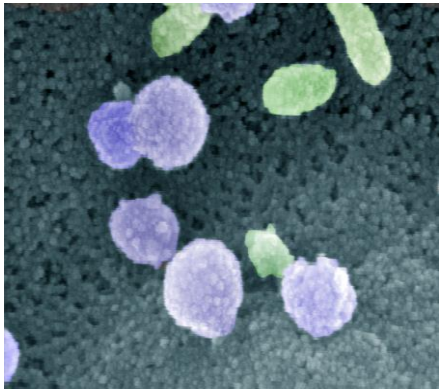
Teunis Geijtenbeek and his group nicely showed that early antiviral responses during infection are beneficial in host control of viral replication also during the latency state (illustrated with untreated patients with HIV-1 who express MAVS (Q198K, S409F).

Observation that inhibition of the DDX3-MAVS blockade in primary human vaginal DCs after HIV-1 infection restored type I IFN responses > suggest that topical therapeutic targeting could be beneficial during sexual transmission of HIV-1.

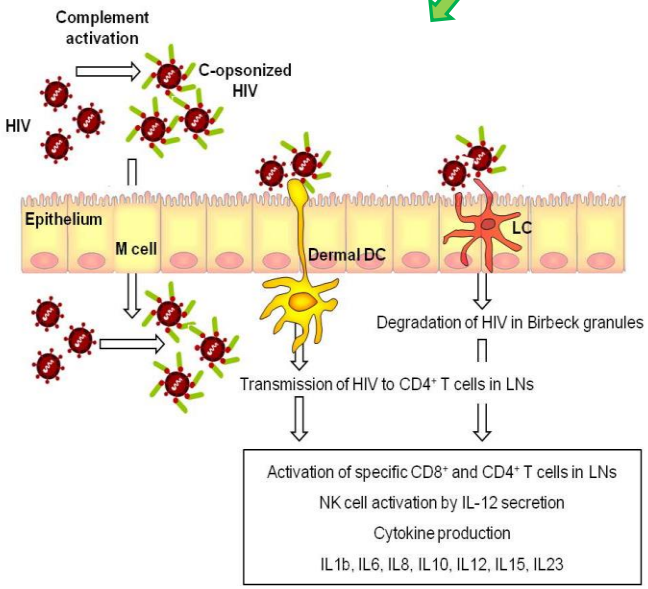
Type I IFN responses are important in acute retroviral exposure, during which time DCs are a prominent target for HIV-1.

Boost of endogenous antiviral immunity in acute exposure or even as prophylactic measure suggested.

BUT: *in vivo* HIV-1 is opsonized with either complement or specific Abs



Acute phase of infection
IA of DCs with HIV-C or HIV



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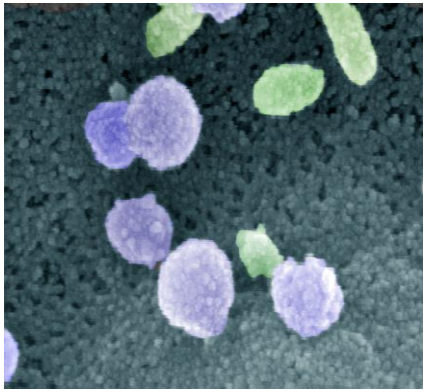
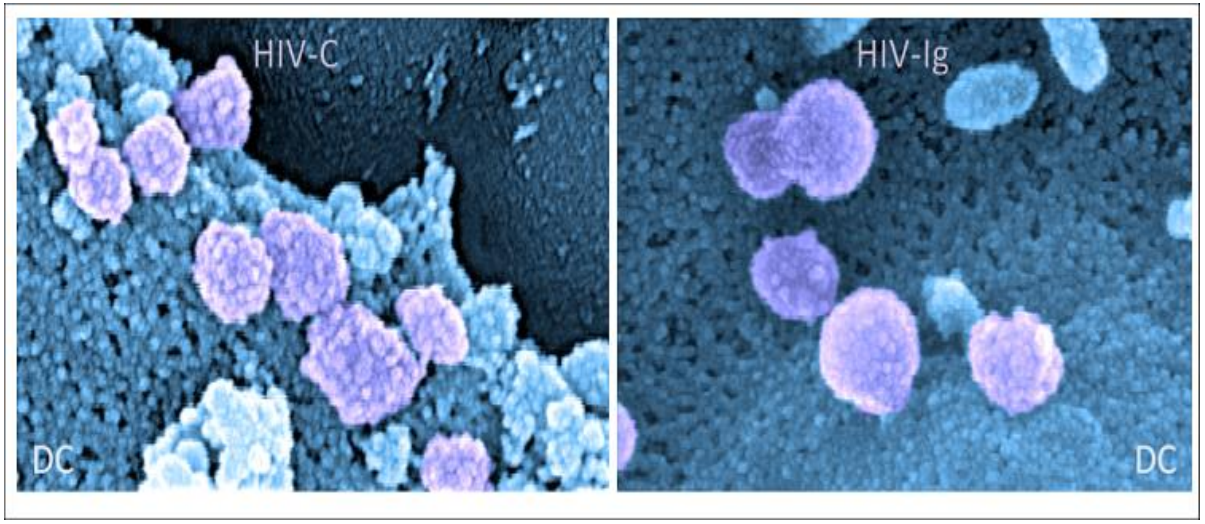


Acute phase of infection
IA of DCs with HIV-C or HIV

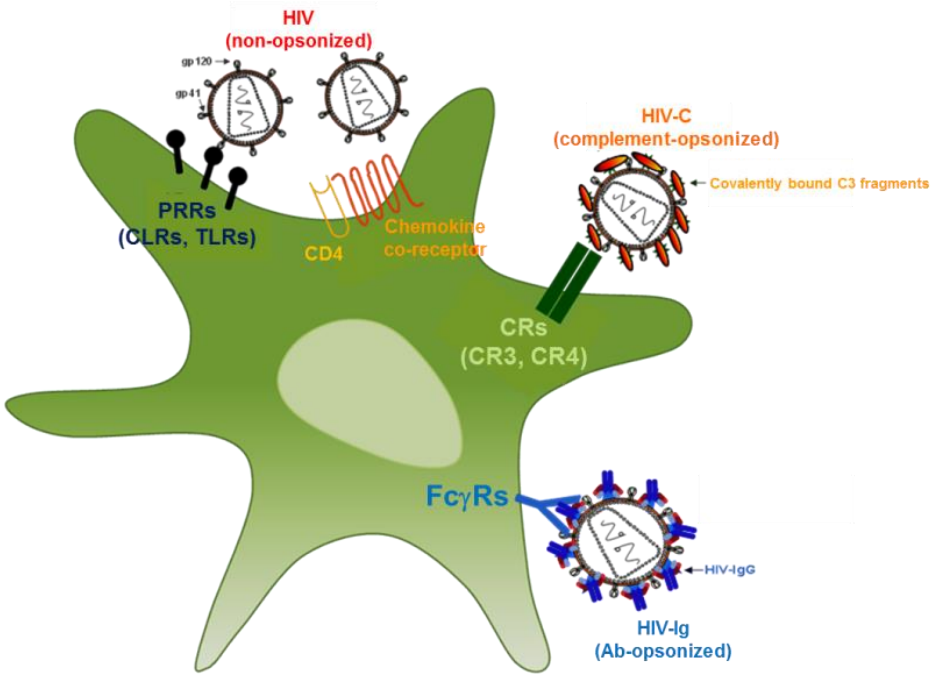
Chronic phase of infection
IA of DCs with HIV-CIg via CRs or FcRs



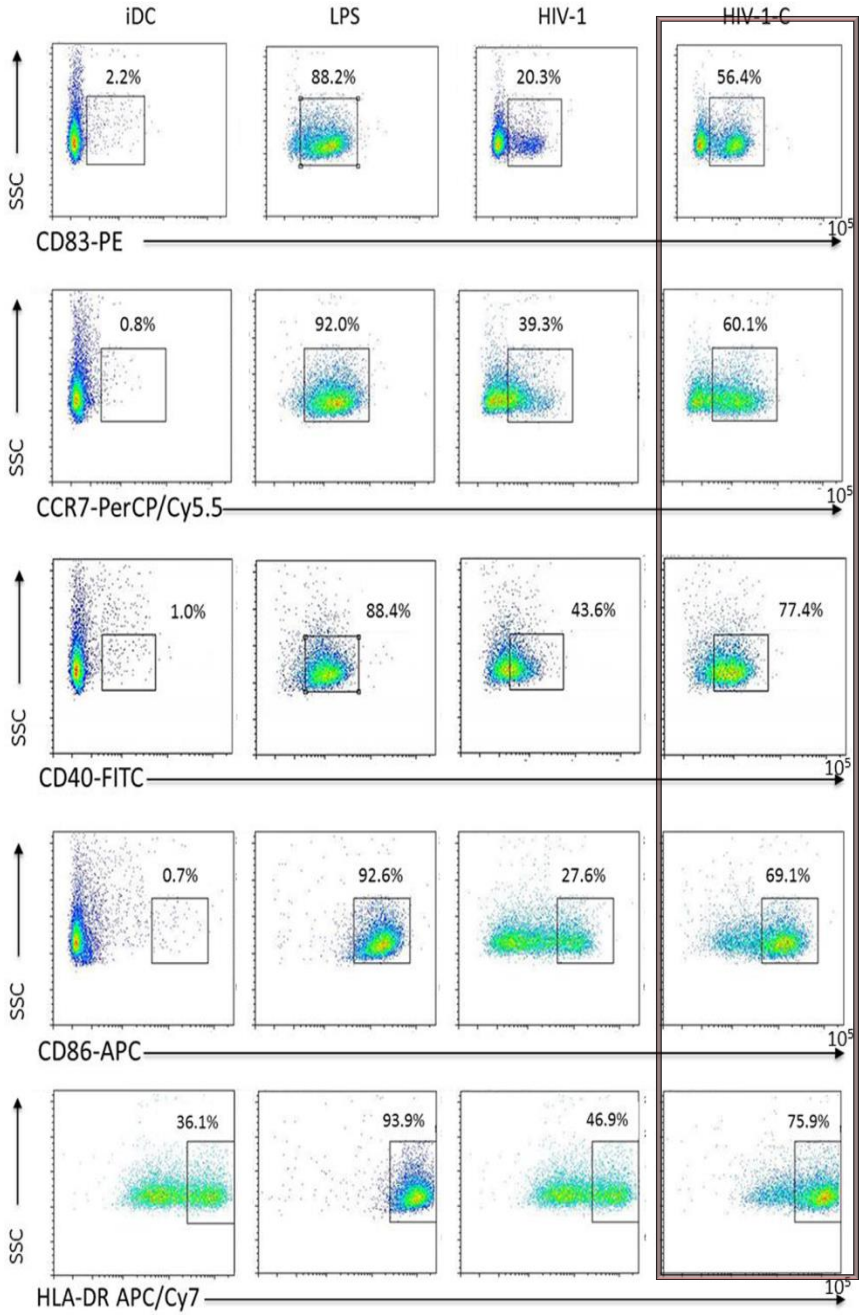
Posch et al., AIDS Res Hum Retroviruses, 2014

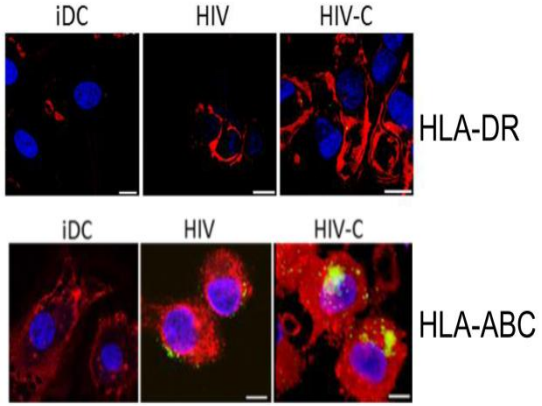
Based on previous findings that

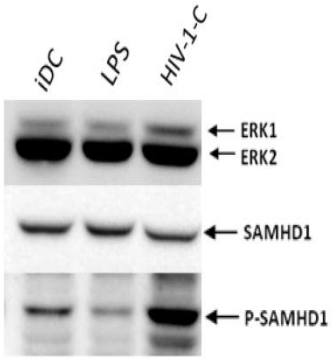
- DCs > innate sensors to specifically inhibit HIV-1 replication (e.g. Manel et al., 2010; Lahouassa et al., 2010; White et al., 2013; Rasaiyaah et al., 2013)
- DCs > only low-level productive infection with HIV-1 (Yan and Liebermann, 2011)
- DCs > non-permissive > viral evasion (Manel and Littman, 2011)
- Type I IFNs are not induced by non-opsonized virus (Gringhuis et al, 2017)



DC FUNCTIONALITY AFTER HIV-C EXPOSURE:: ENHANCING DC MATURATION > COMPARE FIGURE 8 GRINGHUIS ET AL, 2017

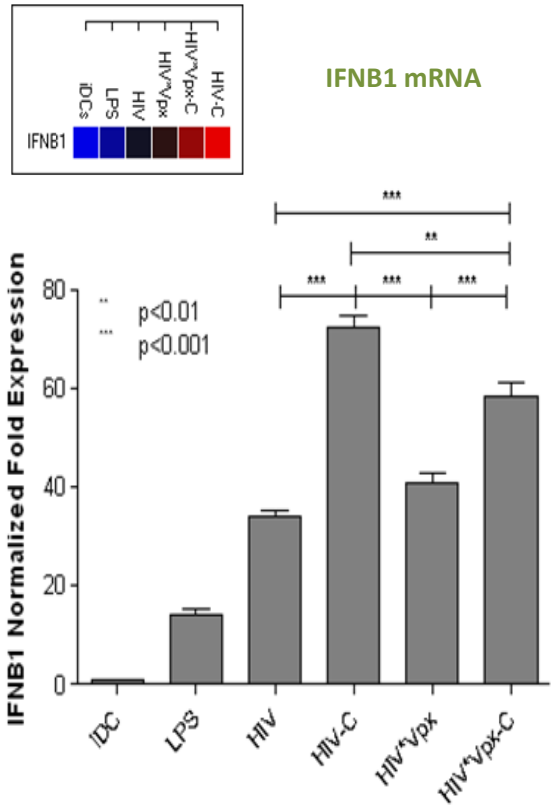


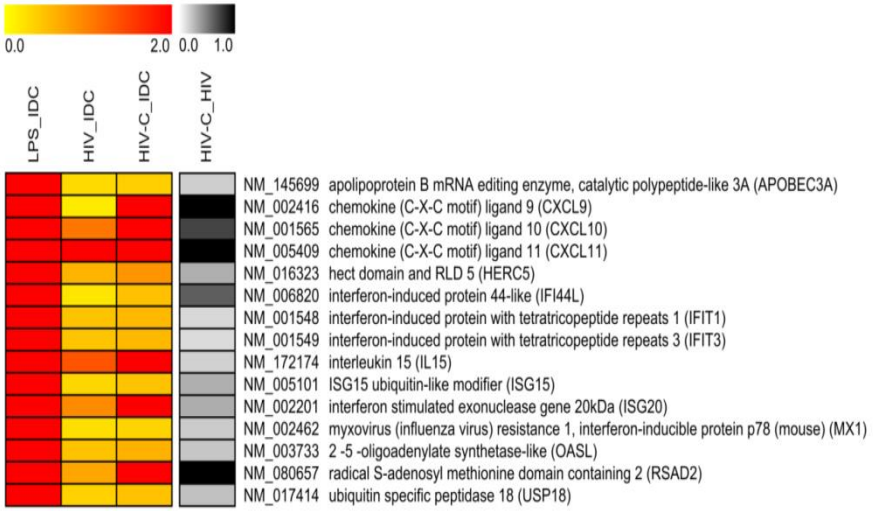




Posch et al., PLoS Pathogens, 2015

DC FUNCTIONALITY AFTER HIV-C EXPOSURE :: INCREASING TYPE I IFN RESPONSES > COMPARE FIGURE 8 GRINGHUIS ET AL, 2017

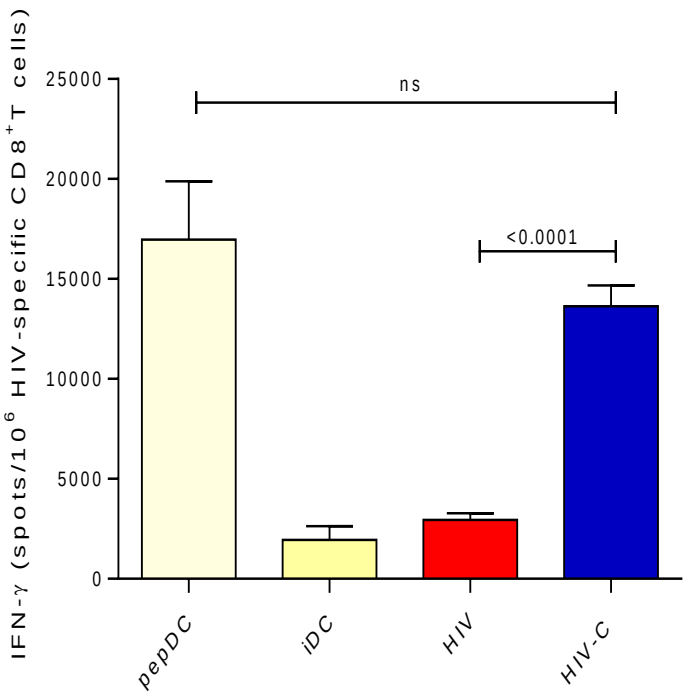




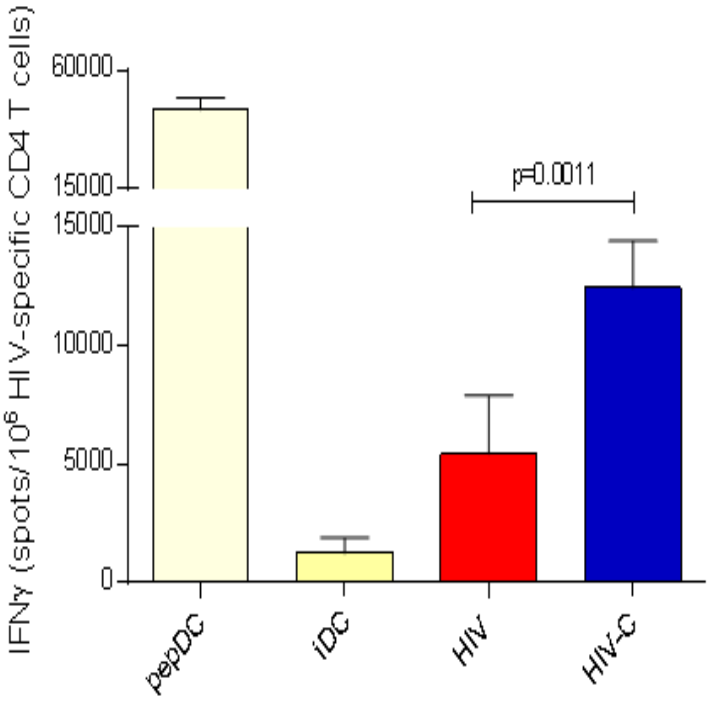
Posch et al., PLoS Pathogens, 2015

DC FUNCTIONALITY AFTER HIV-C EXPOSURE:: EXERTING A HIGHER SPECIFIC TC STIMULATION

HIV-SPECIFIC CD8+ T CELLS

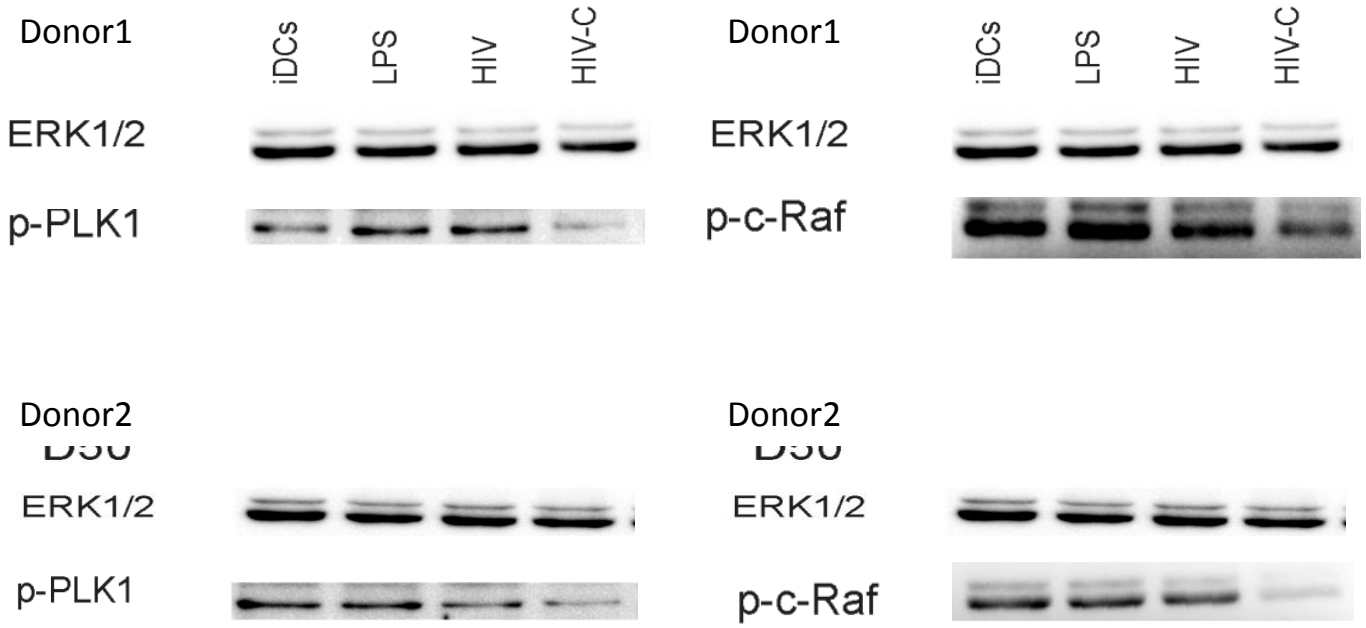


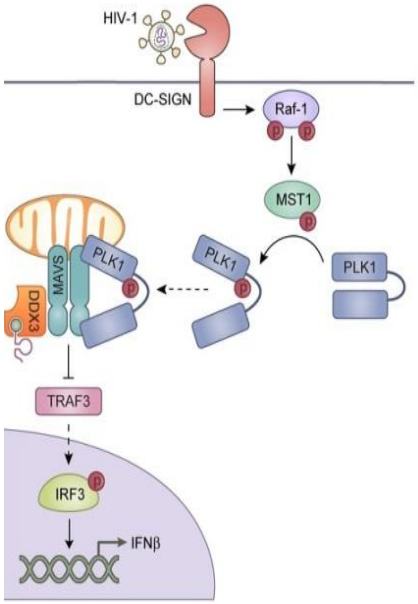
HIV-SPECIFIC CD4+ T CELLS



Posch et al., PLoS Pathogens, 2015

DOWN MODULATION OF SIGNALING PATHWAYS ASSOCIATED WITH MAVS INHIBITION IN HIV-C-DCs



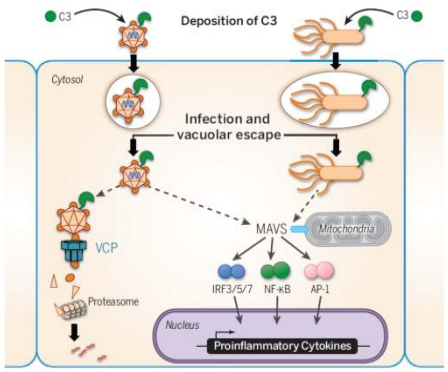


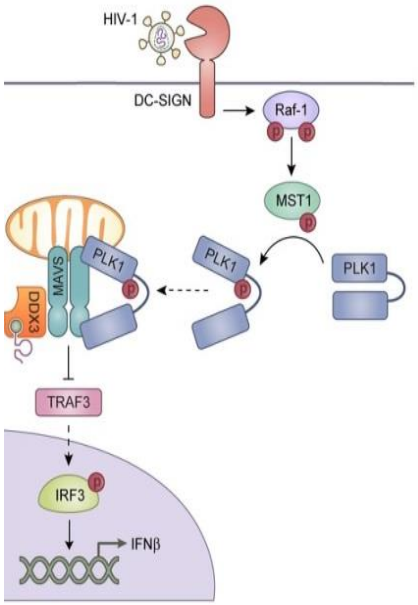
Theo Geijtenbeek,
Nature Immunology 2017

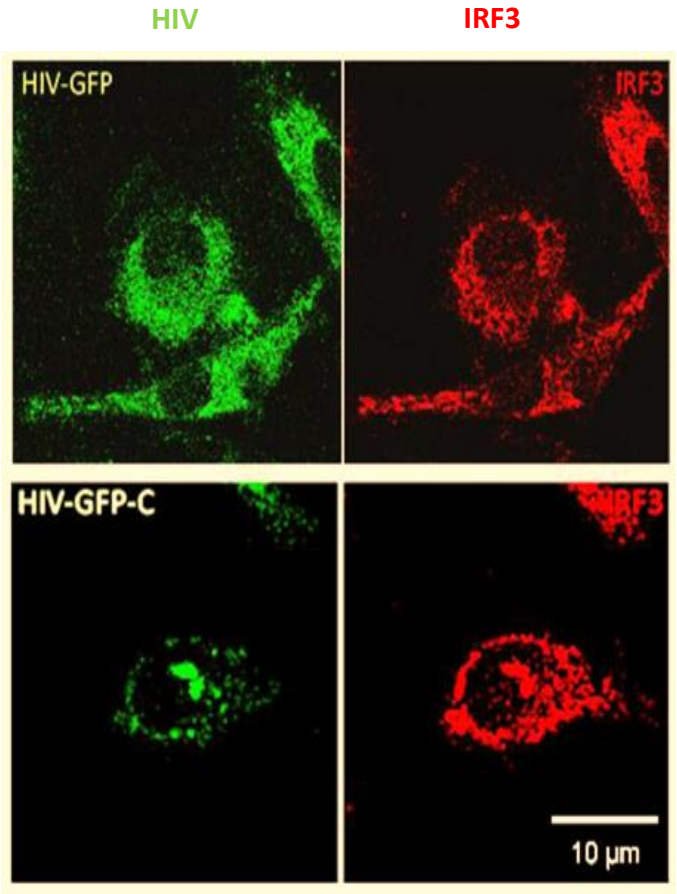
HIV-C down-modulates PLK1 and Raf phosphorylation compared to iDCs, LPS-DCs or HIV-DCs

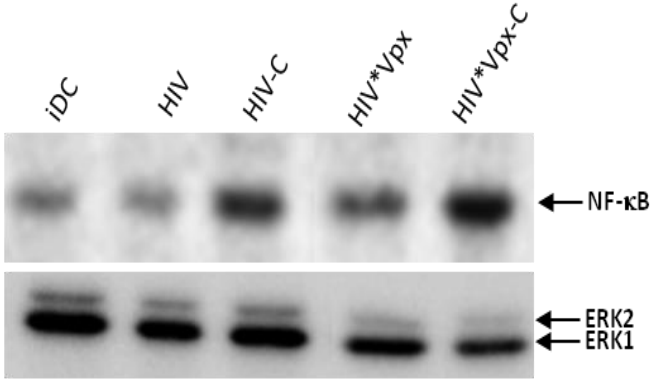
Posch et al., submitted

HIV-C INDUCES MAVS AGGREGATION IN DCs AND SUBSEQUENTLY ALSO IRF-3 AND NFκB ACTIVATION



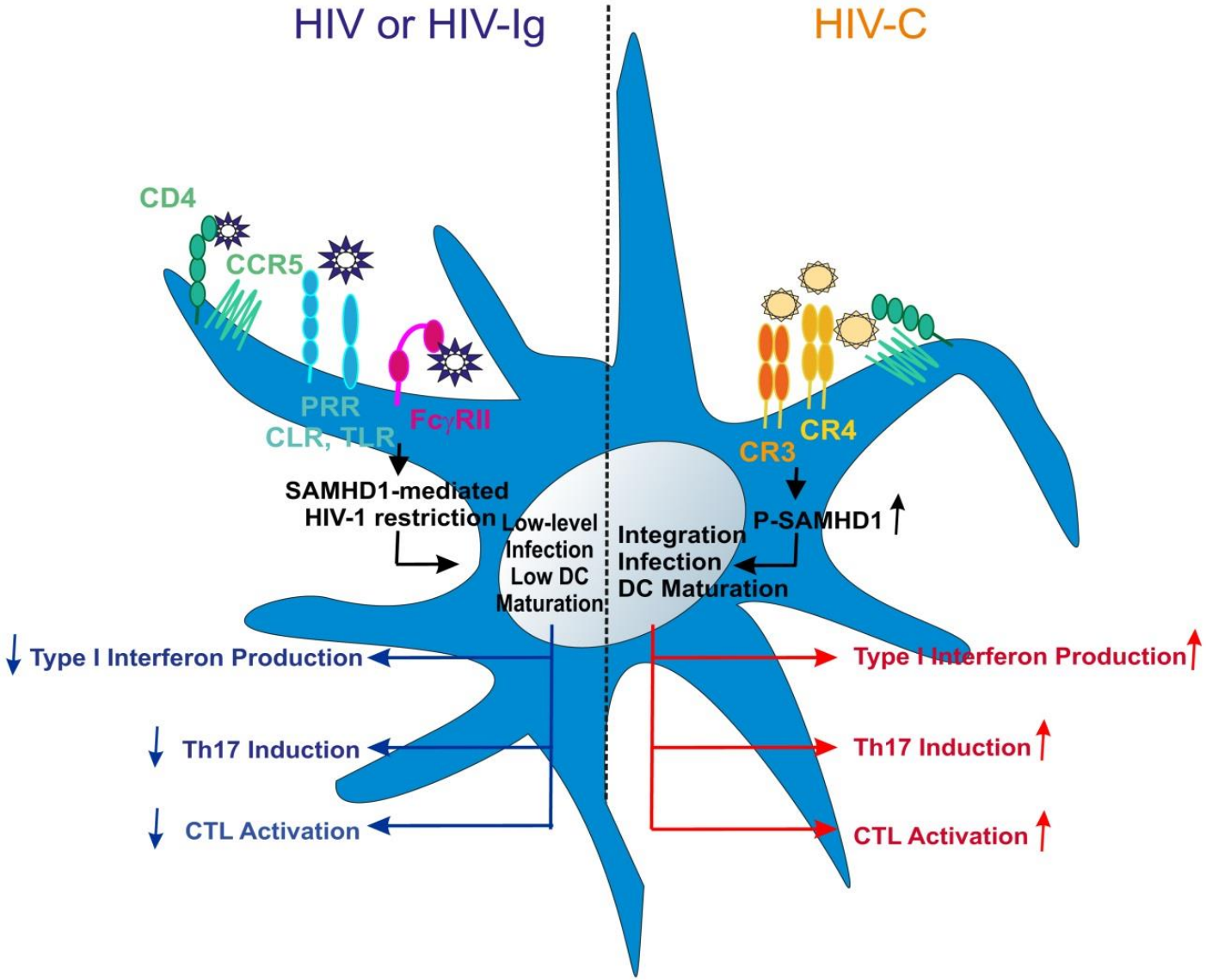






Posch et al., PLoS Pathogens, 2015

GETTING THE RIGHT MOMENT: TARGETING COMPLEMENT FOR NOVEL HIV-1 VACCINATION STRATEGIES



Complement opsonization together with DCs plays a central role in virus control especially during acute phase of infection