



Detecting Infectious HIV in Human Milk

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Question?

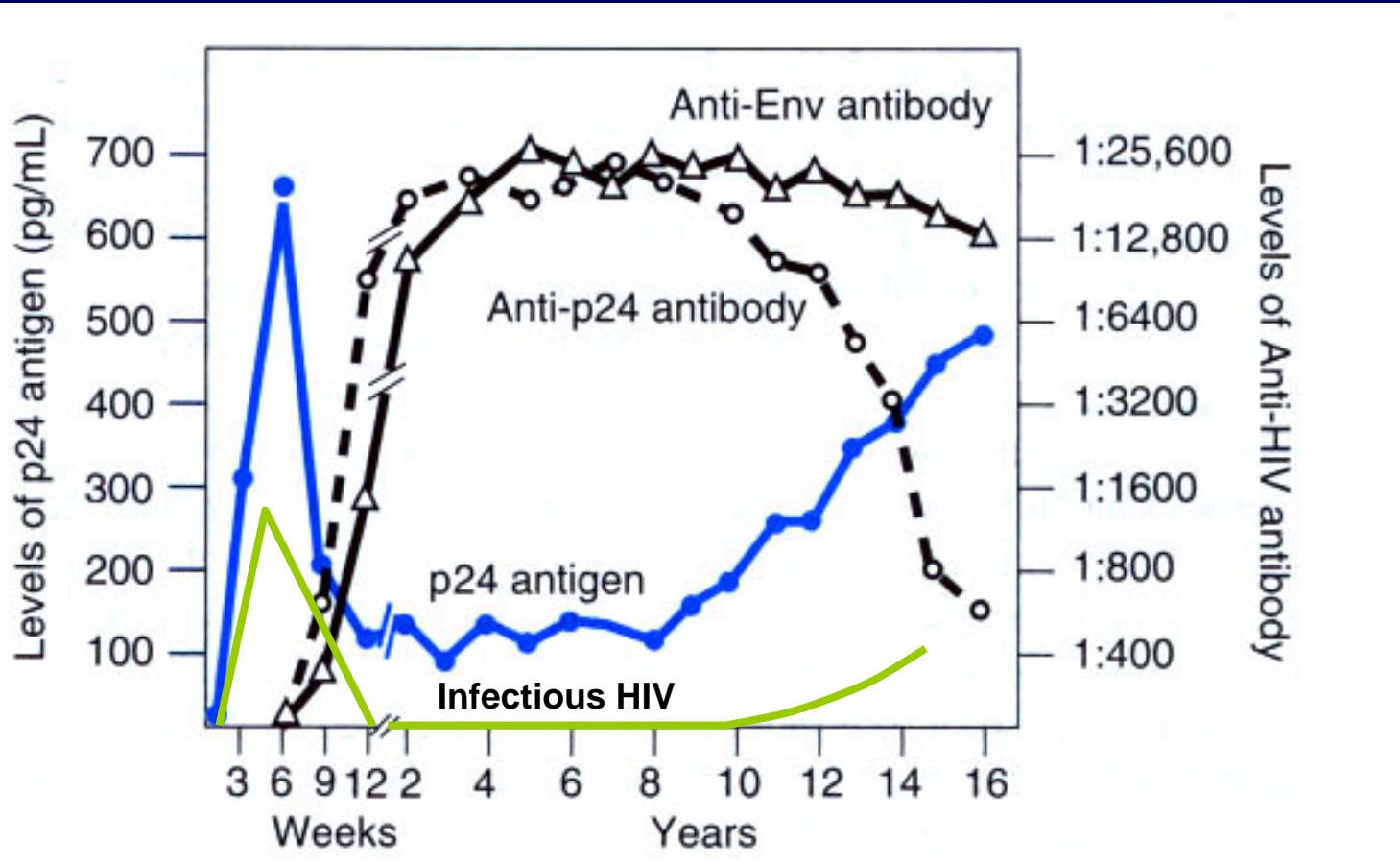
**Does human milk contain
infectious HIV?**



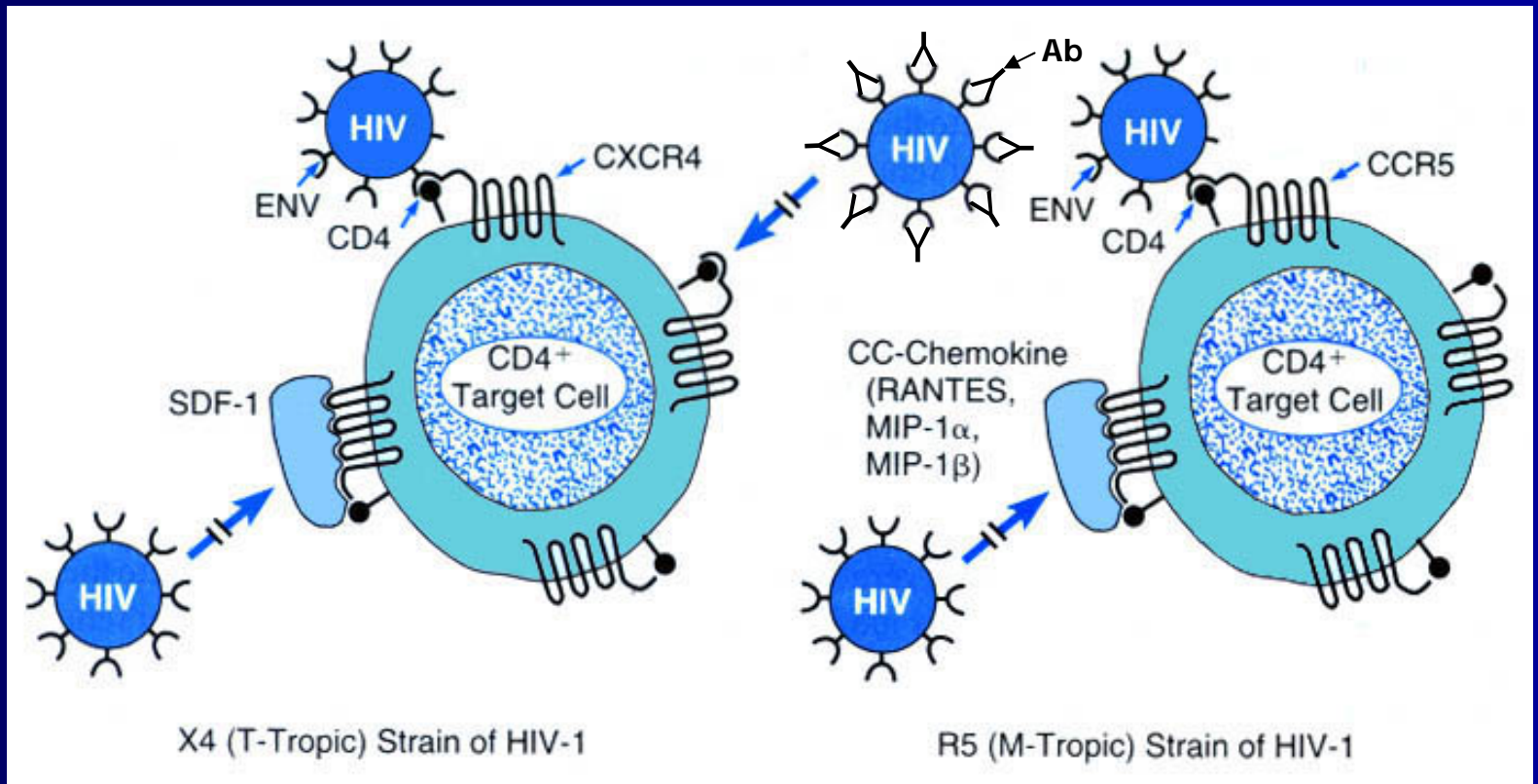
What is known?

- HIV RNA detectable by PCR in milk of 60-90% of HIV + mothers (usually requires testing of multiple samples from each mother).
- Correlation of milk HIV RNA levels with higher plasma HIV loads, lower blood CD4 counts, detection of HIV DNA in maternal genital secretions, and mastitis.
- Milk contains several inhibitors of HIV infectivity (lactoferrin, SLPI, EPO, antibodies)
- Infectious HIV has not been detected.

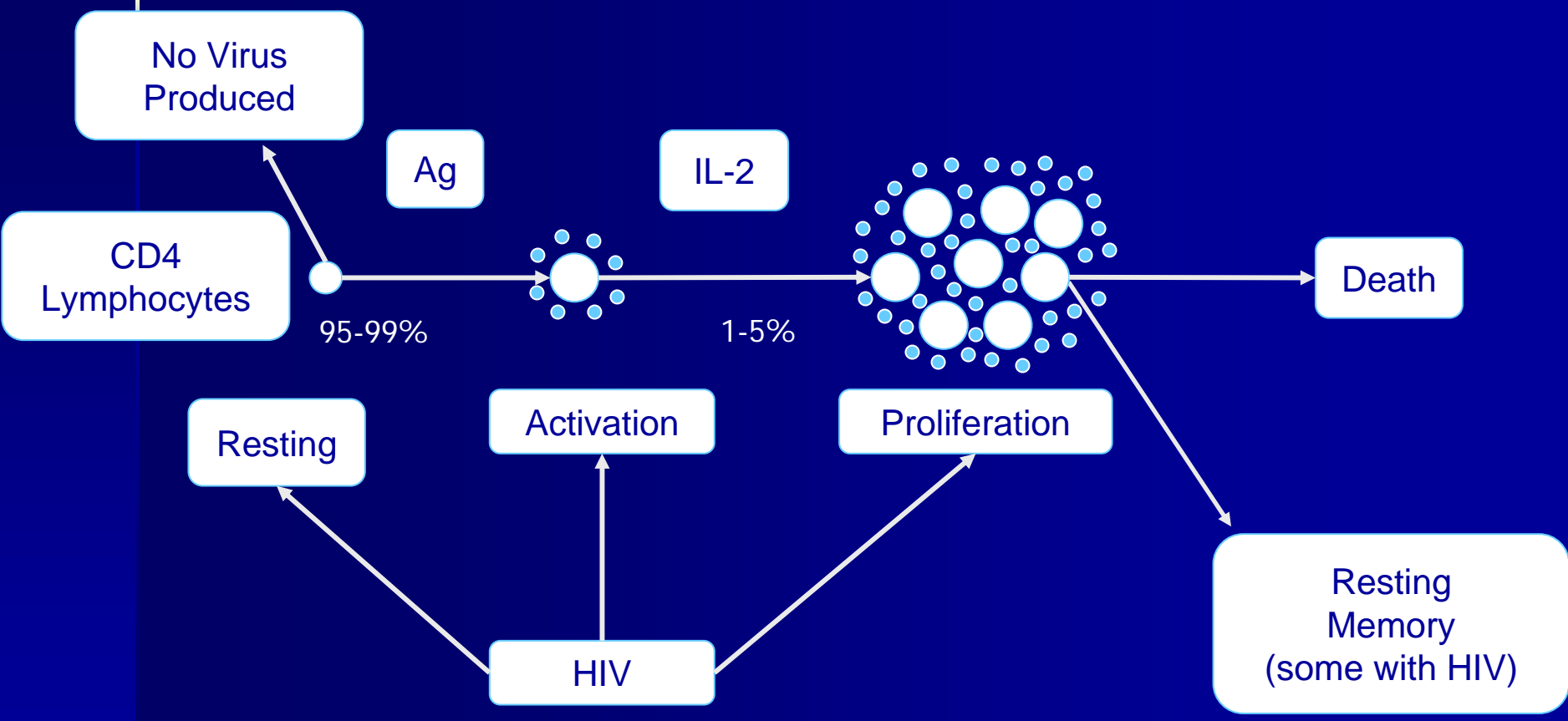
Blood Plasma



How HIV Infection Occurs



HIV Infection in the Body





Human Genes Implicated with Influencing HIV Infection and/or HIV Disease Progression

※ HLA/Tap

※ CCR2B-64I

※ SDF1-3A

※ CCR5 Δ 32

※ Unidentified
genes
conferring
post-entry
restriction in
CD4 T-cells



Summary of Parameters for HIV Infectivity

- HIV quantity in body fluids generally low (blood plasma > milk > genital secretions)
- HIV virions mostly neutralized by antibodies
- B-chemokines made at high levels in local vicinity can inhibit HIV infection.
- 95-99% of all CD4 lymphocytes are resting (not permissive for viral replication).
- Host resistance genes.



Probability of HIV Transmission (per event)

-Kissing	0
-Oral sex	0.0001
-Breast-feeding	0-0.0001
-Vaginal sex	0.0003-0.002
-Anal sex	0.01-0.005



Distribution of Leukocytes in Milk

<u>Total cells/ml</u>	
Colostrum	10^6 - 10^7
Mature milk	10^4 - 10^5
<u>Monocyte-macrophage (%)</u>	
Colostrum	47-66
Mature milk	44
<u>Lymphocytes (%)</u>	
Colostrum	5-11
Mature Milk	2
<u>Lymphocyte subsets (% of total lymphocytes)</u>	
B cells (total)	7-35
T cells (total)	50-88
CD4+	43
CD8+	48
NK	9


Levels ($\mu\text{g/ml}$) of Immunoglobulins in Human External Secretions

Fluid	IgA	IgG	IgM	IgA1 (%)	IgA2	pIgA	mIgA
Tears	80-400	trace-16	0-18	59	41	95	5
Nasal secretions	70-846	8-304	0				
Parotid saliva	15-39 120-319	0.4 2-5	0.4				
Whole saliva	194-206	42	64	63	37	96	4
Bronchoalvcolar fluid	3	13	0.1	67	33	72	28
Colostrum Milk	12.340 470-1632	100 40-168	610 50-340	52-65	35-48	90-95	5-10



Problems with studies attempting to detect Infectious HIV in human milk

1. HIV virions in milk fluid are likely to be neutralized by antibodies (non-infectious)
2. Presence of other inhibitory factors in milk (lactoferrin, SLPI)
3. HIV-infected cells present in low numbers
 - Blood: 1-10% of CD4 cells abortively infected
 - 0.001% of CD4 cells productively and latently infected.
4. Sample storage or preparation not compatible with maintaining cells healthy.



How Studies to Detect Infectious HIV should be performed:

1. Fresh milk samples, taken immediately to lab.
2. Centrifuged to separate cells from fluid portion.
3. Density centrifugation of cellular components to separate mononuclear cells from other cell types.
4. Magnetic bead sorting to retain CD4 lymphocytes and monocytes.
5. Mononuclear cell culture PHA-stimulated and grown in IL-2 containing media.
6. Add fresh PHA-CD4 blasts at 3 weeks.
7. Monitor weekly for HIV-p24 by Ag-capture EIA or PCR.