

XV International AIDS Conference

Pathogenic Mechanisms of HIV Disease: The Role of Viral Replication and Immune Activation

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Diseases**

**National Institutes of Health
Bethesda, Maryland, USA**

July 15, 2004



Viral Pathogenesis



**Pathogenesis of
HIV Disease**

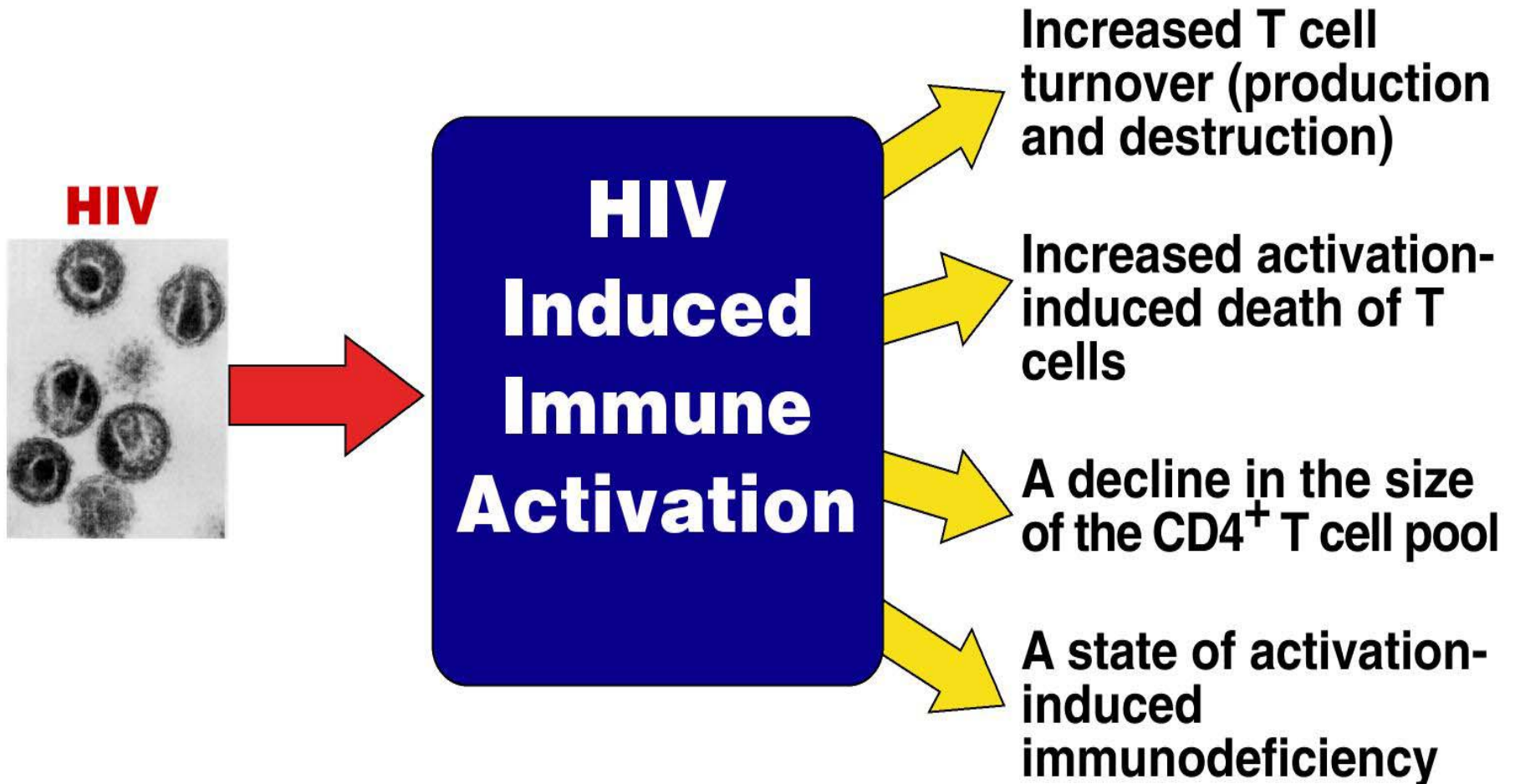


Immunopathogenesis

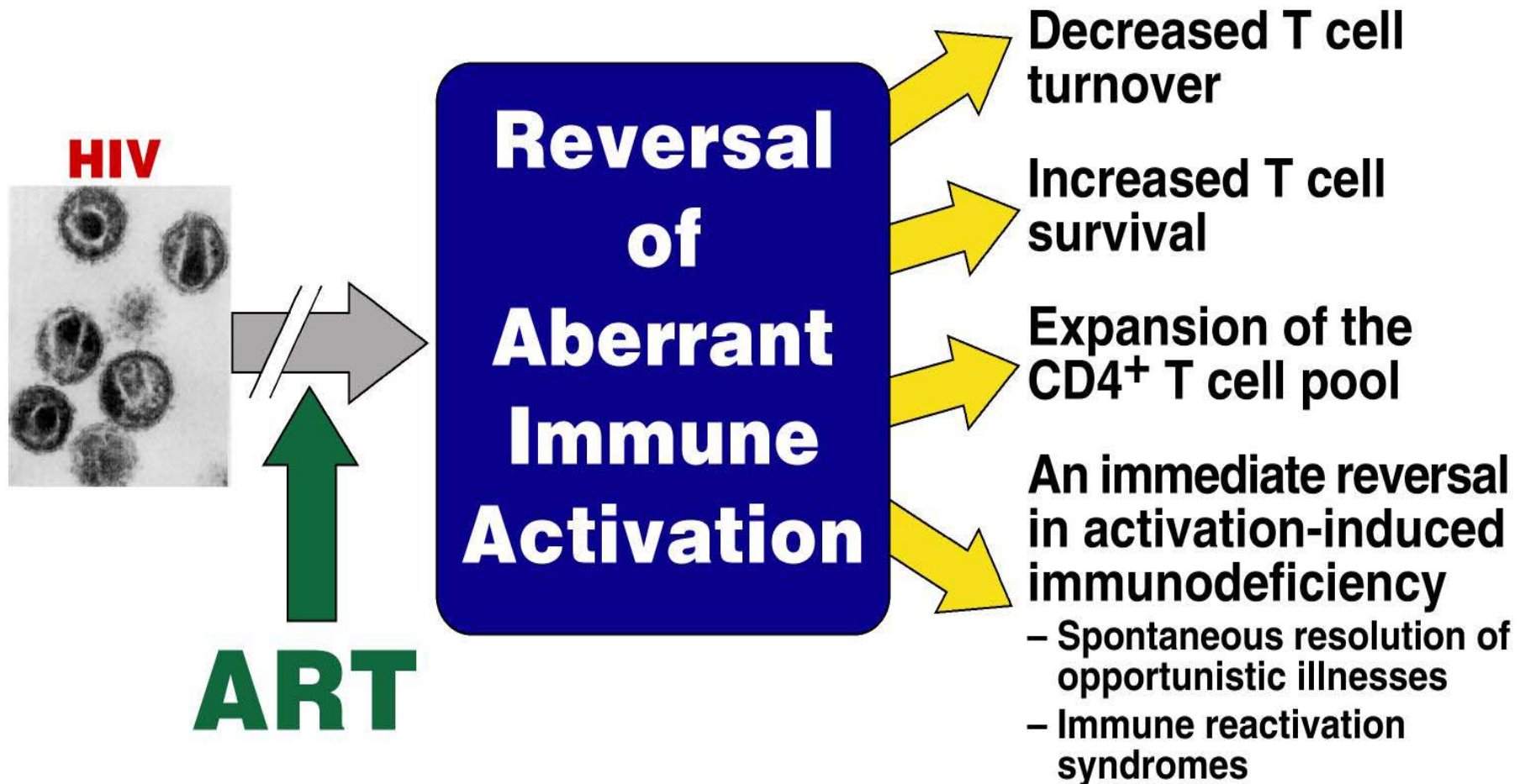
Immune Activation Drives HIV Pathogenesis



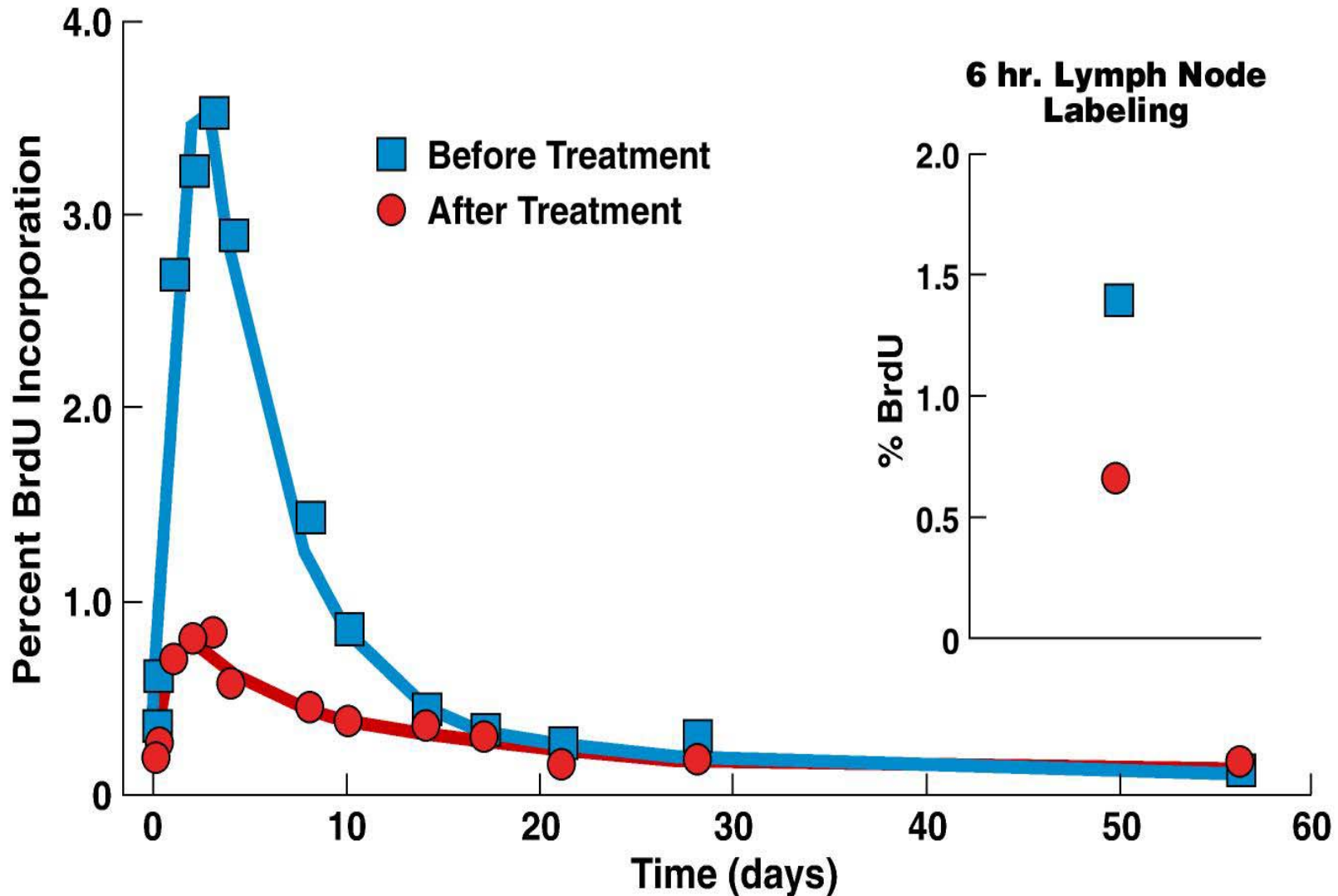
HIV Infection Leads to a State of Generalized Immune Activation



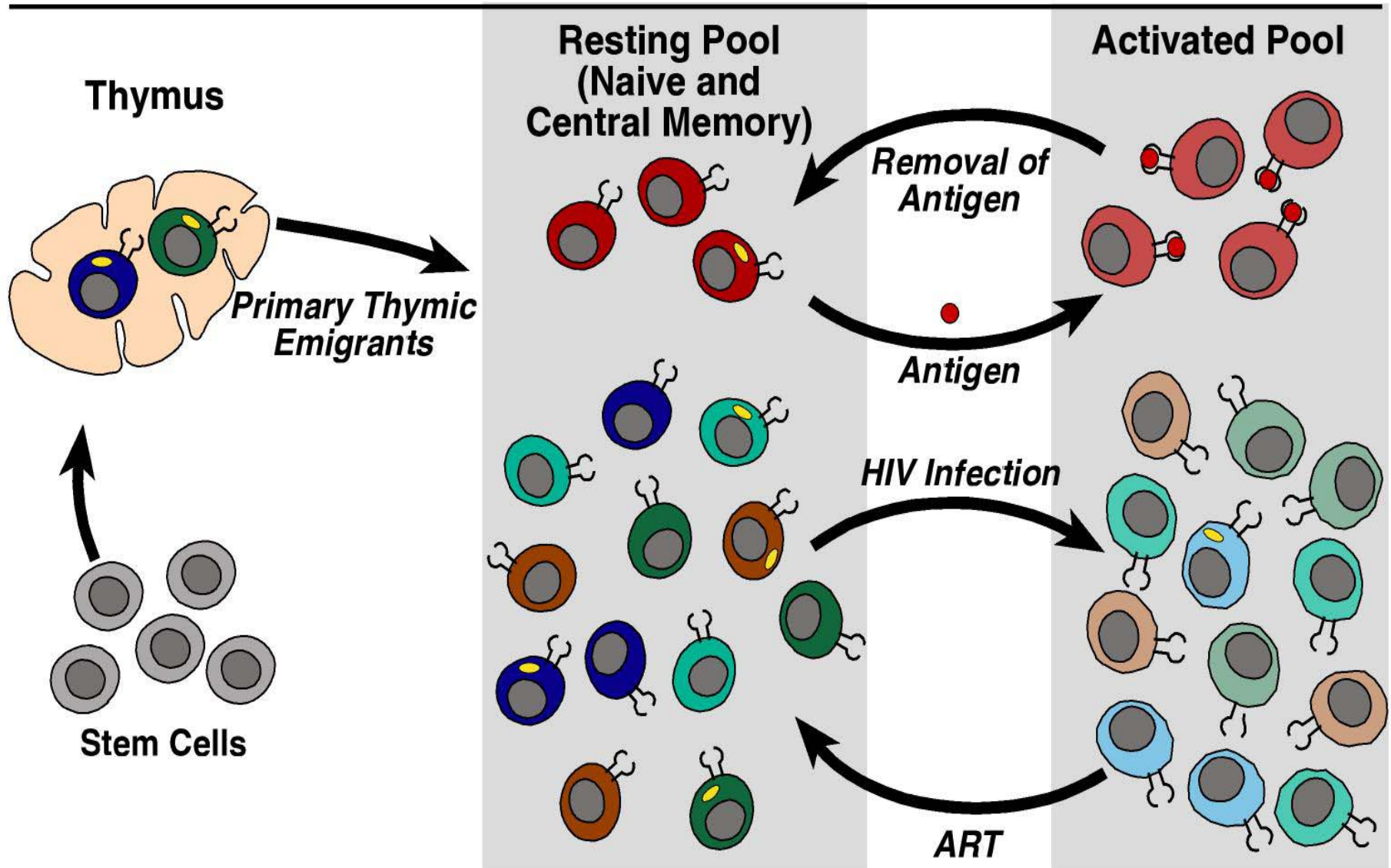
The Impact of ART on HIV-Associated Immune Activation



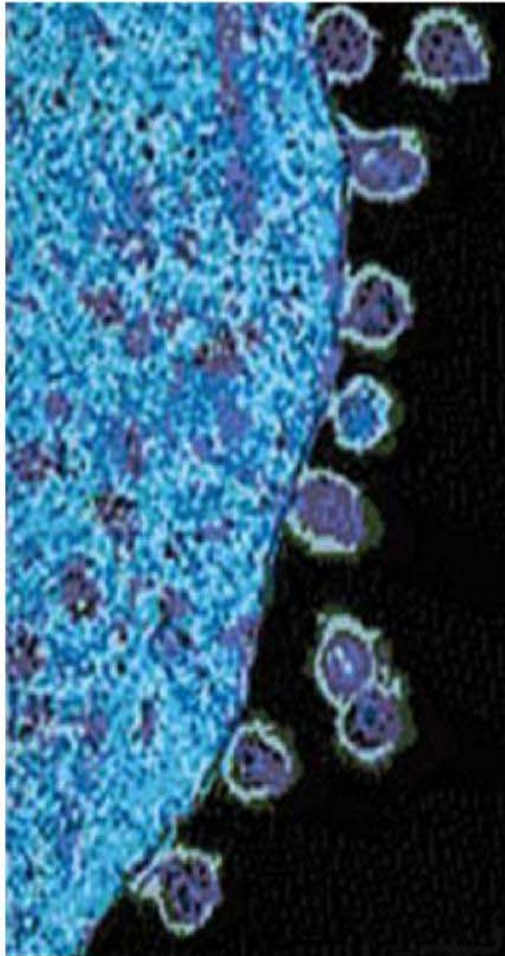
Changes in CD4⁺ T Cell Production Following Initiation of Antiretroviral Therapy



Impact of Antigen and HIV Infection on the CD4⁺ T Cell Pool



Impact of Viral Replication and Viremia on Lymphocyte Subsets in HIV-Infected Individuals



**CD4+ T Cell
Reservoirs**

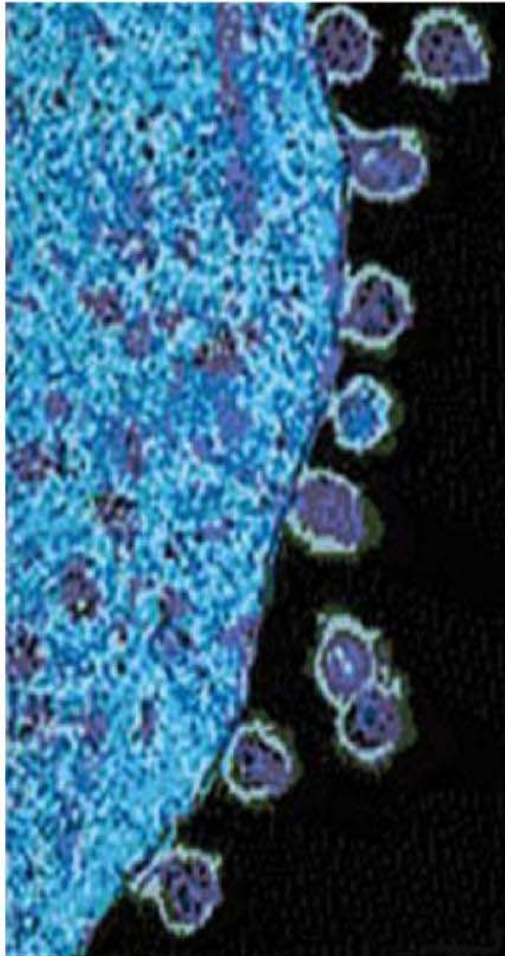


B Cells



NK Cells

Impact of Viral Replication and Viremia on Lymphocyte Subsets in HIV-Infected Individuals



**CD4+ T Cell
Reservoirs**



B Cells



NK Cells



Presence of an Inducible HIV-1 Latent Reservoir During Highly Active Antiretroviral Therapy

Tae-Wook Chun, Lieven Stuyver, Stephanie B. Mizell, Linda A. Ehler, Jo Ann M. Mican, Michael Baseler, Alun L. Lloyd, Martin A. Nowak, and Anthony S. Fauci

PROCEEDINGS
OF THE
NATIONAL ACADEMY OF SCIENCES
OF THE UNITED STATES OF AMERICA

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INCLUDES: FROM THE ACADEMY FEATURING GERMAN-AMERICAN FRONTIERS OF SCIENCE



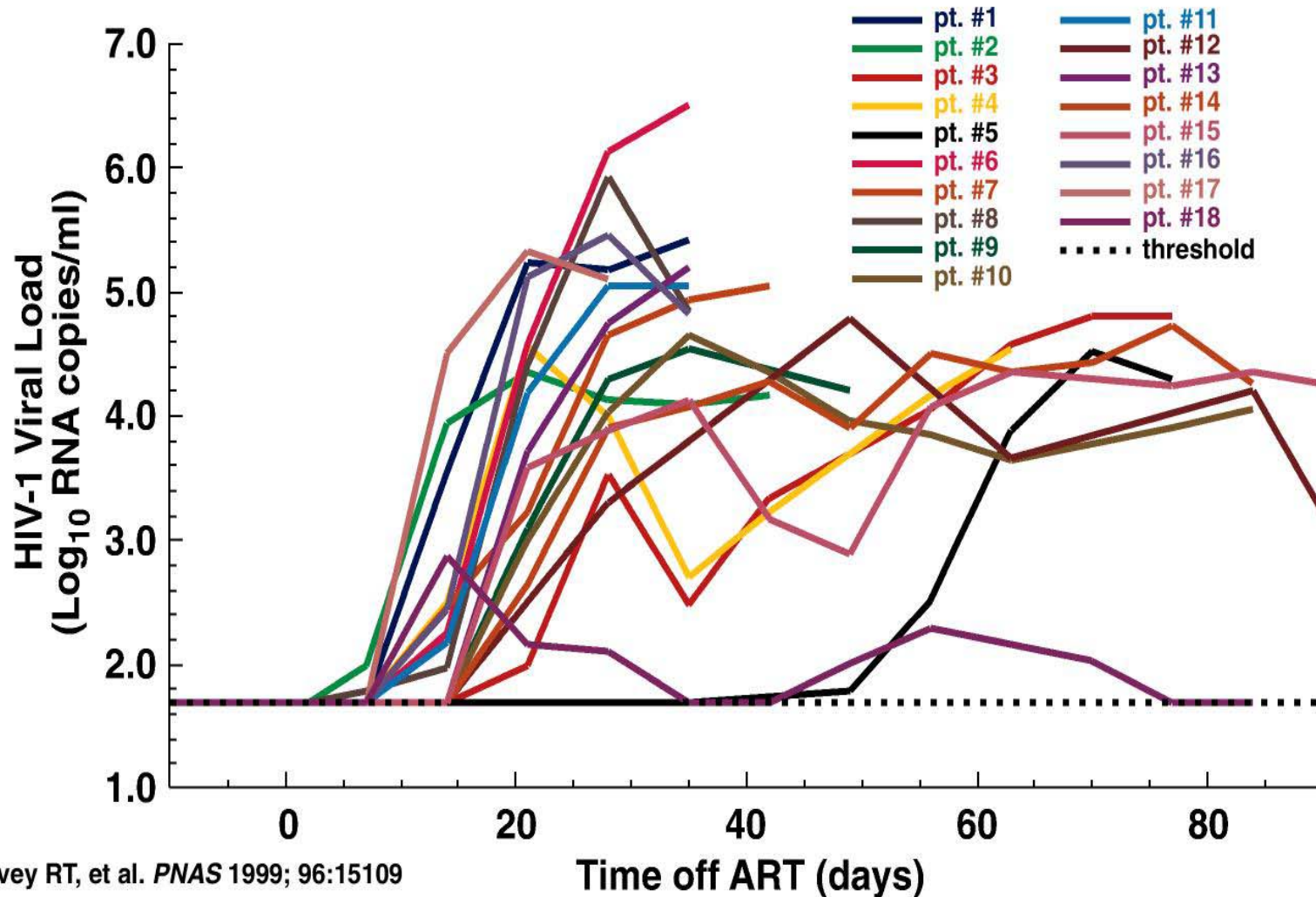
Recovery of Replication-Competent HIV Despite Prolonged Suppression of Plasma Viremia

Joseph K. Wong, Marjan Hezareh, Huldrych F. Günthard, Diane V. Havlir, Caroline C. Ignacio, Celsa A. Spina, Douglas D. Richman

Identification of a Reservoir for HIV-1 in Patients on Highly Active Antiretroviral Therapy

Diana Finzi, Monika Hermankova, Theodore Pierson, Lucy M. Carruth, Christopher Buck, Richard E. Chaisson, Thomas C. Quinn, Karen Chadwick, Joseph Margolick, Ronald Brookmeyer, Joel Gallant, Martin Markowitz, David D. Ho, Douglas D. Richman, Robert F. Siliciano

Viral Relapse Following Discontinuation of ART



Importance of Viremia in Replenishment of the HIV Latent Reservoir



Effect of interleukin-2 on the pool of latently infected, resting CD4⁺ T cells in HIV-1-infected patients receiving highly active anti-retroviral therapy

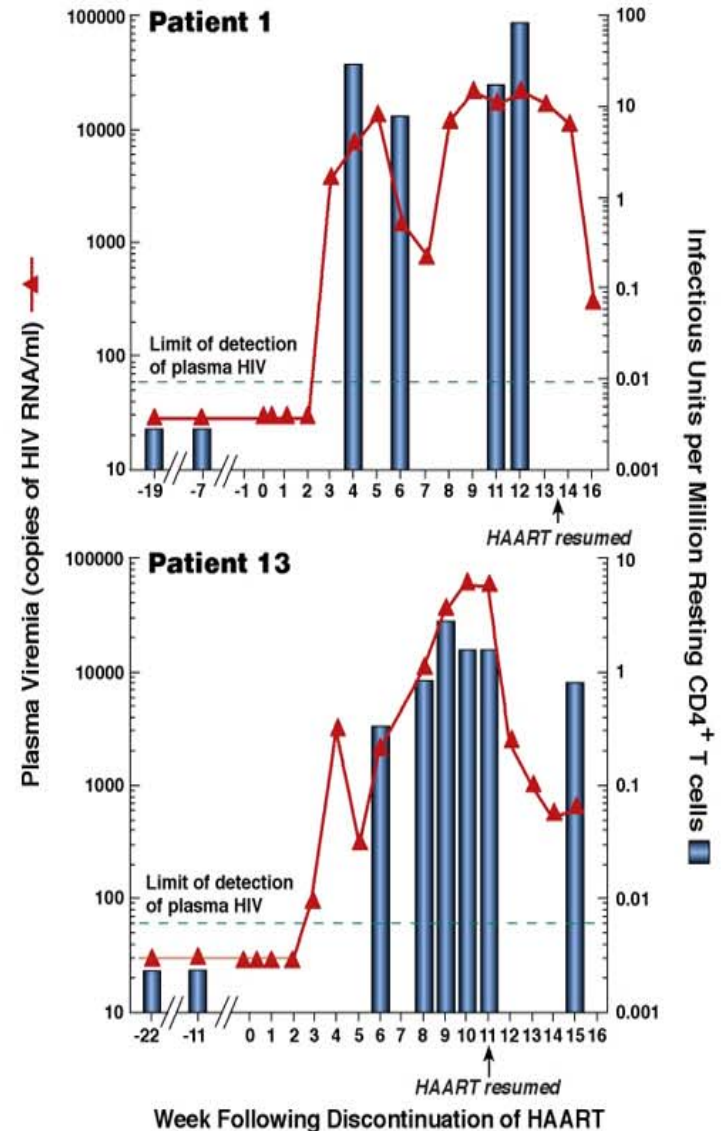
Tae-Wook Chun, Richard T. Davey Jr,
Delphine Engel, H. Clifford Lane,
Anthony S. Fauci

Answers to the atypical measles puzzle

Autoimmune disease in mice lacking serum amyloid protein

Extracellular matrix proteins protect cancer cells

HAART, IL-2 and the eradication of HIV-1





The
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Established in 1812 as THE NEW ENGLAND JOURNAL OF MEDICINE AND SURGERY

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Quantifying Residual HIV-1 Replication in Patients Receiving Combination Antiretroviral Therapy

Linqi Zhang, Bharat Ramratnam, Klara Tenner-Racz, Yuxian He, Mika Vesanen, Sharon Lewin, Andrew Talal, Paul Racz, Alan S. Perelson, Bette T. Korber, Martin Markowitz, and David D. Ho

VOLUME 9 NUMBER 6 JUNE 2003
www.nature.com/naturemedicine

nature medicine

Long-Term Follow-Up Studies Confirm the Stability of the Latent Reservoir for HIV-1 in Resting CD4⁺ T Cells

Janet D. Siliciano, Joleen Kajdas, Diana Finzi, Thomas C. Quinn, Karen Chadwick, Joseph B. Margolick, Colin Kovacs, Stephen J. Gange and Robert F. Siliciano

PNAS

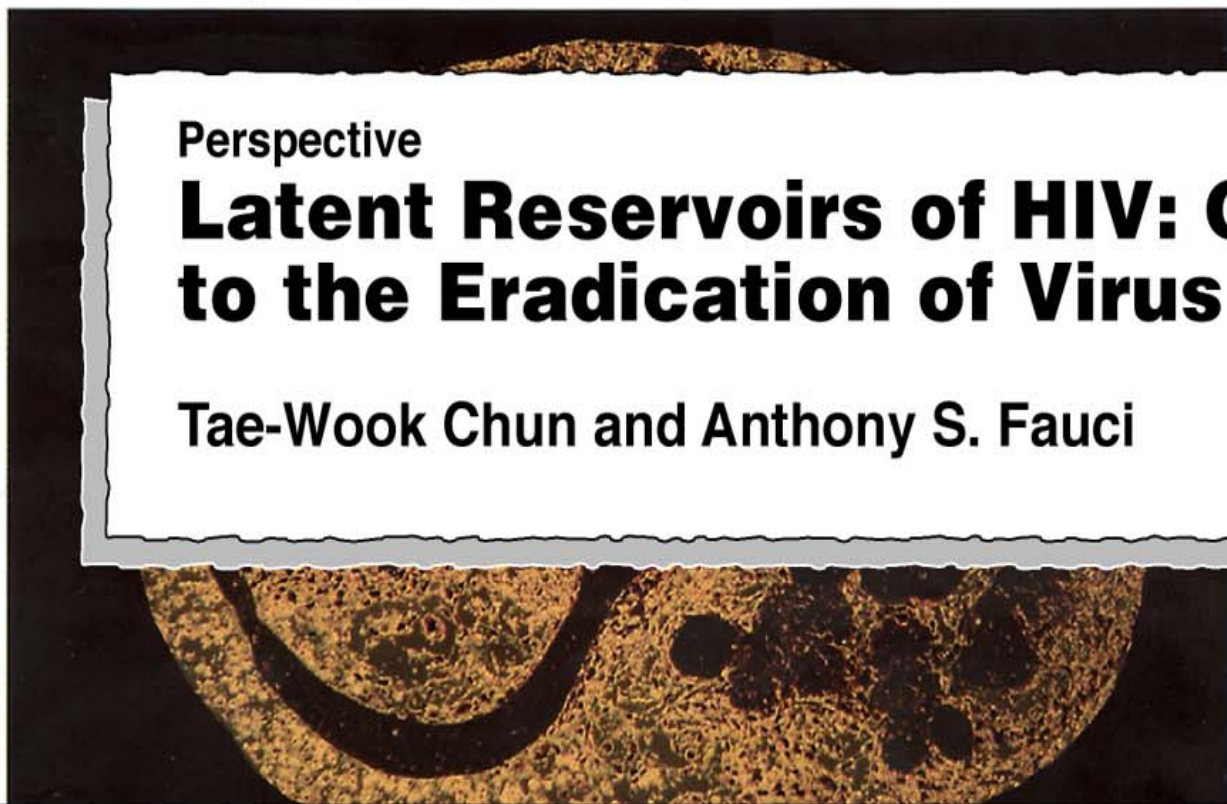
Proceedings of the National Academy of Sciences
of the United States of America

September 28, 1999 | vol. 96 | no. 20 | pp. 10945–11688 | www.pnas.org

Perspective

Latent Reservoirs of HIV: Obstacles to the Eradication of Virus

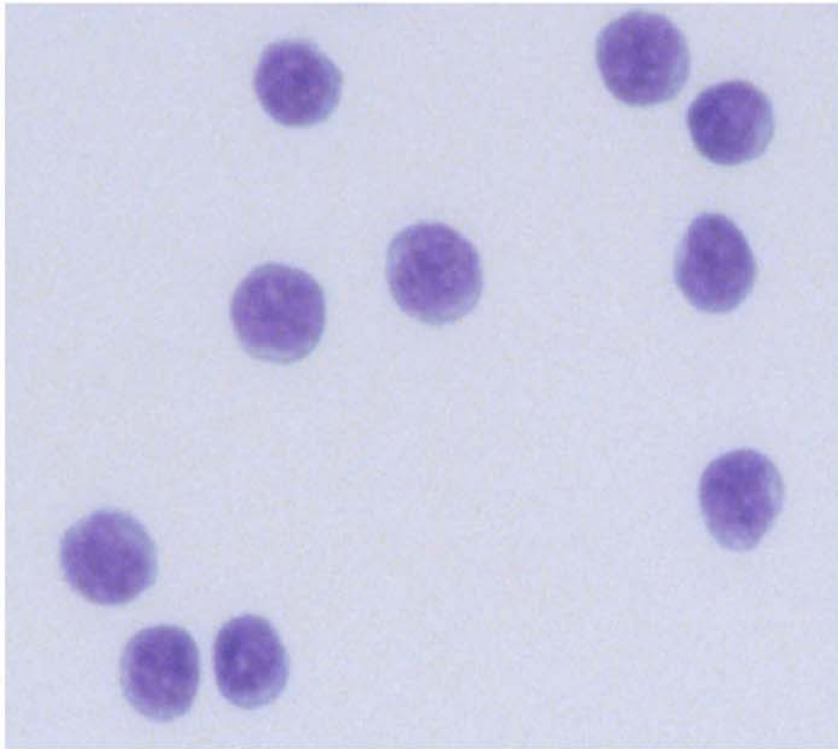
Tae-Wook Chun and Anthony S. Fauci



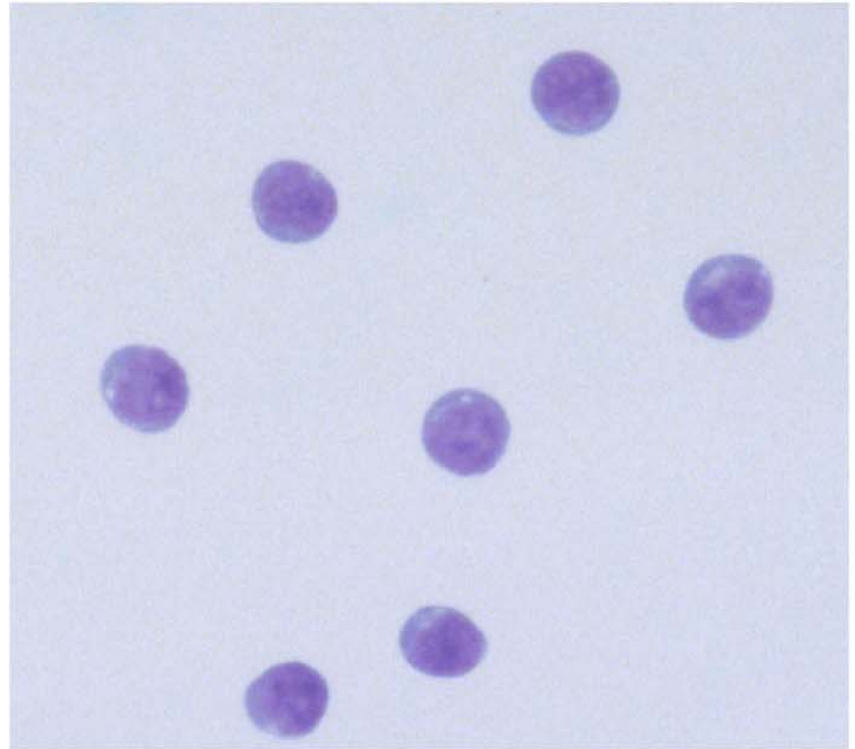
The Resting CD4⁺ T Cell Reservoir of HIV in Viremic Versus Aviremic Individuals

Morphology of Resting CD4⁺ T Cells

Aviremic Patient

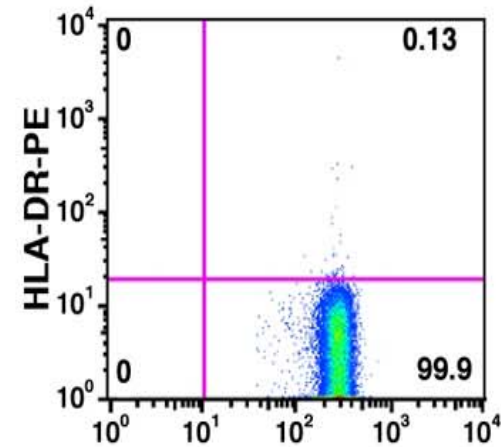
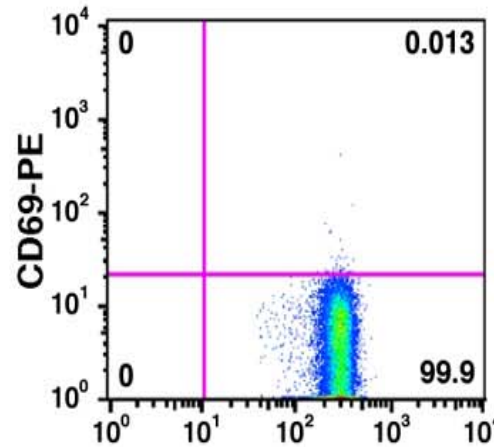
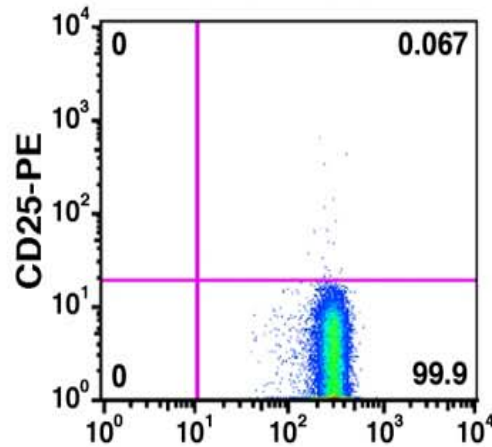


Viremic Patient

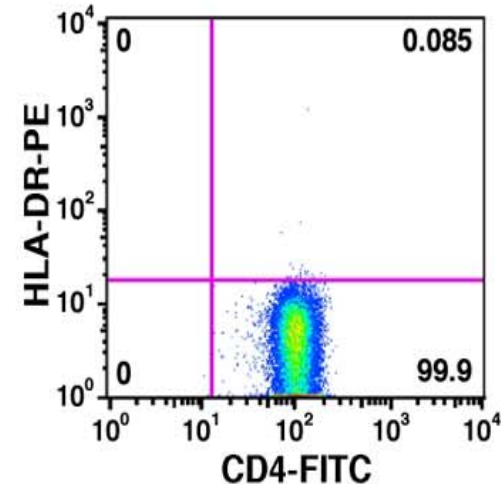
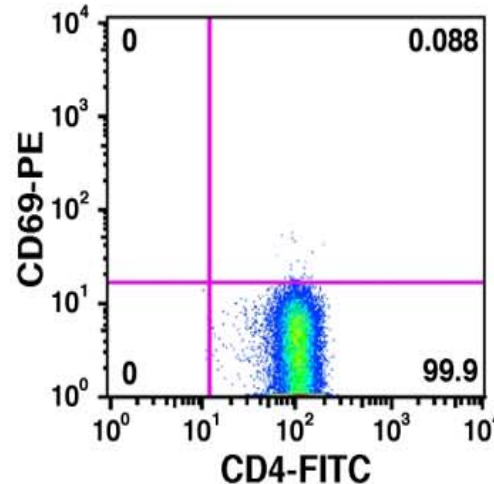
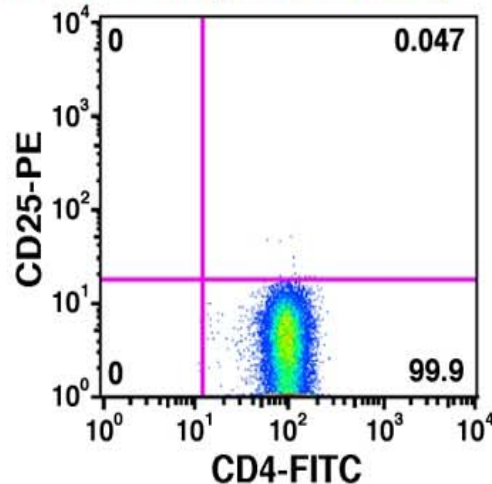


Resting CD4⁺ T Cell Reservoir for HIV in Viremic versus Aviremic Individuals is Phenotypically Identical

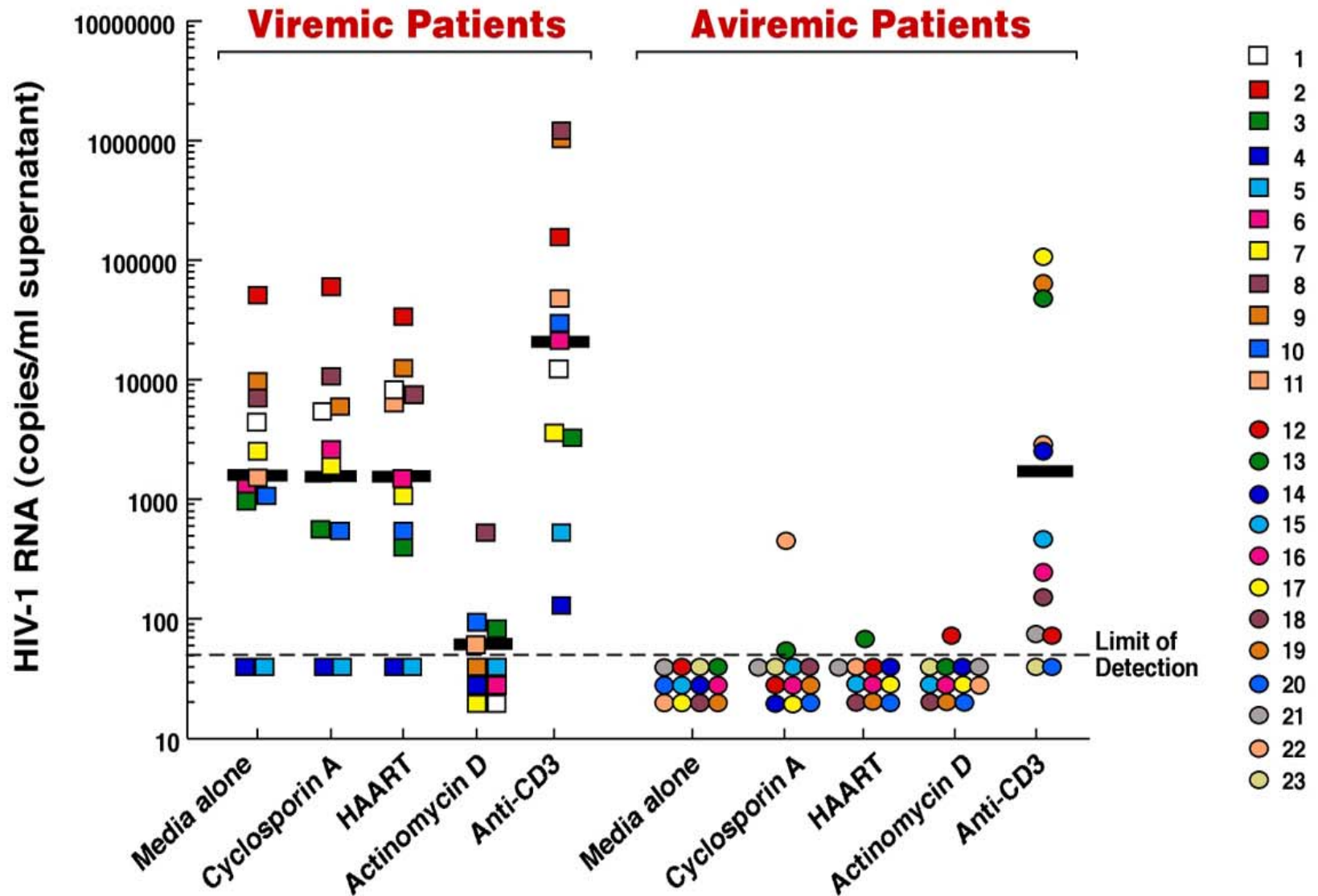
Patient 4 (Viremic)



Patient 22 (Aviremic)

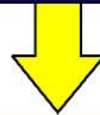


Levels of Cell-Free HIV Virions Released by Latently Infected, Resting CD4⁺ T Cells from Viremic and Aviremic Patients



DNA Microarray Analysis of Resting CD4⁺ T Cells from Aviremic and Viremic HIV-Infected Patients

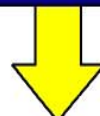
Isolation of RNA from resting CD4⁺ T cells



Synthesis and hybridization of cRNA onto Affymetrix Human Genome U95A Oligonucleotide Array (12,600 genes)

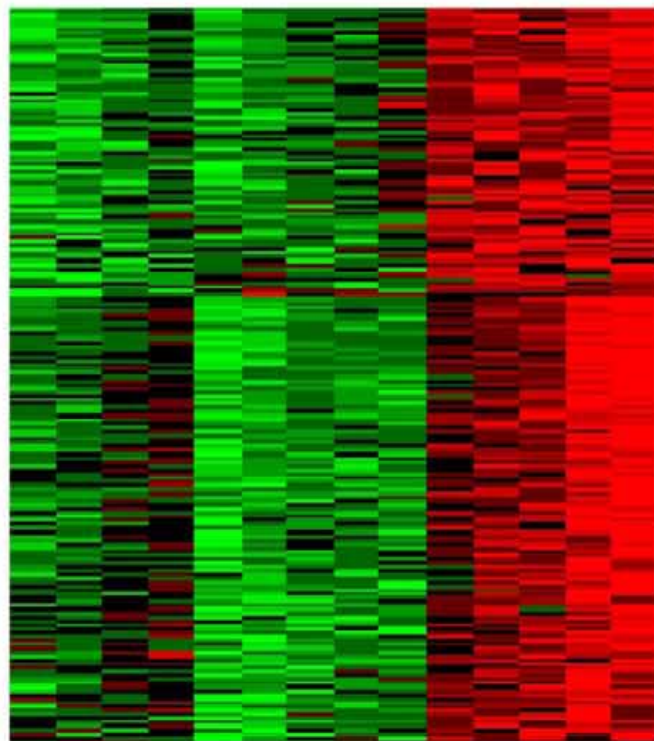


Analysis of data using Significant Analysis of Microarrays algorithm



Output of data using descriptive categories of genes using literature-mining algorithm

DNA Microarray Analysis of Resting CD4⁺ T Cells from Aviremic and Viremic Patients and Normal Donors



A B C D | 16 13 18 15 23 | 10 9 2 6 7

HIV-negative donors | Aviremic patient | Viremic patient



Cluster 4

Cluster 5

Transcription Regulators (p=0.0004)

Identifer	Symbol	Locus	Name
131_at	TAF11	11510	TAF11 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 29kDa
1643_a_at	MTA1	11510	metastasis associated 1
326_c_at	HMGB1	11510	high-mobility group nucleosome-binding domain 1
31787_at	TBP1	8619	TBP-like 1
31876_at	FUZF3	8599	far upstream element (FUSE) binding protein 3
33835_at	MZF1	25794	myristic acid-dependent transcription oncogene homolog 1 (avian)
32841_at	ZNF5	7455	zinc finger protein 5 a cellular leucine/tyrosine nucleic acid binding protein
33381_at	NCOA3	8202	nuclear receptor coactivator 3
34358_at	HDCA2	3086	histone deacetylase 2
34497_at	TFIIA	1789	nuclear transcription factor II, beta
35156_at	MYCN	4613	v-myb myelocytomatosis viral related oncogene, neuroblastoma derived (avian)
35802_at	ASH2L	1070	ash2 (absent, small, or homeotic) like (Drosophila)
36186_at	GTF3A	2571	general transcription factor IIIA
37352_at	SF100	6572	nuclear antigen SF100
37620_at	TAF12	6930	TAF12 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 20kDa
37620_at	TFIIB	4331	nuclear transcription factor II, beta
37974_at	MSL3L1	10843	male-specific lethal 3 like 1 (Drosophila)
38055_at	HMG202	3148	high-mobility group box 2
38305_at	FOI2	3289	pre-B-cell leukemia transcription factor 2
38345_at	CEP1	11094	centrosomal protein 1
38426_at	TAF11	8982	TAF11 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 29kDa
38426_at	ACT2	1772	actin, cytoplasmic 2
39191_at	TCE1	6909	transcription elongation factor B (SII), polypeptide 2 (18kDa, elongin B)
398_c_at	RLN1	861	runt-related transcription factor 1 (acute myeloid leukemia 1, smt1 oncogene)
39826_at	TCE1G1	10815	transcription elongation regulator 1 (CA150)
39826_at	RLN2	8440	runt-related transcription 2 (Drosophila)
40791_at	POLR2A	5430	polymersase (RNA) II (DNA directed) polypeptide A, 20kDa
41360_at	CTCFB	1637	CTCF-HCT1 transcription complex, subunit B
41851_at	MYB	4522	v-myb myeloblastosis viral oncogene homolog (avian)
453_at	SMARCC2	8601	SMARCC2 related, matrix associated, actin dependent regulator of chromatin, subfamily c, member 2
891_at	YY1	7529	YY1 transcription factor

RNA Processing/Modification (p=0.0000005)

Identifer	Symbol	Locus	Name
31950_at	PABPC1	26936	poly(A) binding protein, cytoplasmic 1
32592_at	GRIP1	2505	G-rich RNA sequence binding factor 1
32781_at	PFP4	8599	serine/threonine protein kinase PFP4 homolog
32798_at	NCBP2	22916	nuclear cap binding protein subunit 2, 20kD
33945_at	HNF1PH1	3187	heterogeneous nuclear ribonucleoprotein H1
34167_at	DOV5	1655	RNA helicase, DKO
34753_at	SP3A1	10291	splicing factor 3a, subunit 1, 100kD
35136_at	P15-2	55916	typhoid cell protein P15-2
35997_at	LMNB	11157	lamin B
36654_s_at3181	HNF1A2B1	11157	heterogeneous nuclear ribonucleoprotein A2B1
36913_at	SLBP	7884	sterile-like (histone) binding protein
36985_s_at11340	DIP-2	10295	Drosophila doublesex interacting protein 2
39010_at	SPF30	10295	splicing factor 30
39827_at	NONO	4581	non-POU domain containing, octamer binding
39828_s_at8570	H1-SPF1	8159	H1-type splicing regulatory protein
39925_at	HNF1P1	3180	poly(A) binding protein, nuclear 1
39945_at	HNF1P2	3180	heterogeneous nuclear ribonucleoprotein II
39975_at	HNF1P3	10256	heterogeneous nuclear ribonucleoprotein III
40085_s_at1	HCA7651	27292	human cytosolic arginine transferase
40453_s_at	SPF155	8430	splicing factor, arginine/serine-rich 5
41191_T_at	HNF1PH2	3189	heterogeneous nuclear ribonucleoprotein H2
41192_s_at	GD3P	10146	Rise G1 Phase activating protein

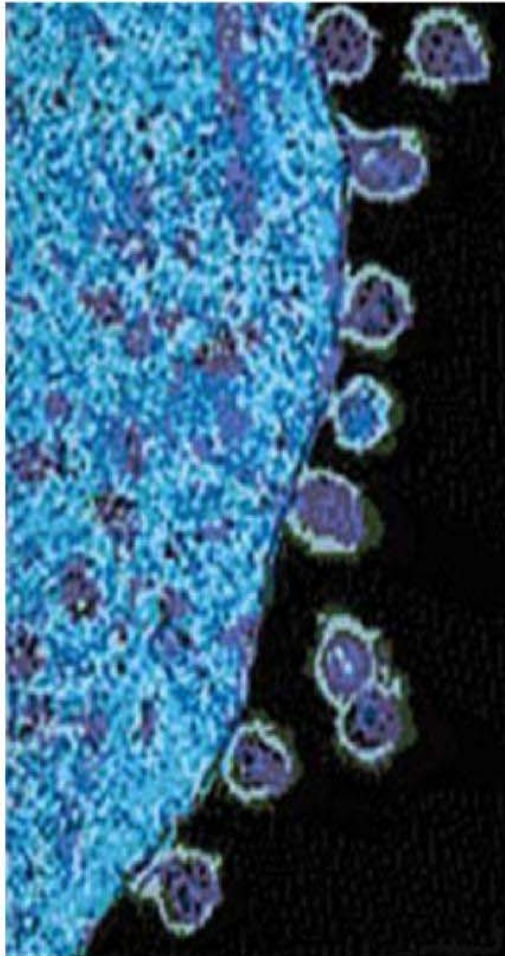
Protein Trafficking/Vesicle Transport (p=0.00002)

Identifer	Symbol	Locus	Name
32179_s_at	SHAP2B	8773	synaptosomal-associated protein, 29kD
32342_at	SEC13-like1	6196	vesicle transport-related protein
33810_at	RAH10	25256	SEC24 related gene family, member A
34196_at	SEC24A	10932	SEC24 related gene family, member A
34370_at	ARH11	3172	aradin 1
34796_at	TRAM	25471	translational chain-associated membrane protein
34870_at	DPM1	8513	dactyl phosphate mannosyltransferase polypeptide 1
37352_s_at8615	FTL1	1115	vesicle docking protein
37352_at	RAB28	3269	RAB28, member RAS oncogene family
37727_T_at	RCH2	3945	reticulocalbin 2, EF-hand calcium binding domain
37795_at	ASB2	433	analogous protein receptor 2
37956_at	GGA3	23128	guanine nucleotide exchange factor 3
38074_at	AP3D1	1175	adaptor-related protein complex 3, sigma 1 subunit
38325_at	BG1	12668	brain 1A-inhibited guanine nucleotide-exchange protein 1
38918_at	GCE2L2	8670	ganglioside M2 receptor complex member 2
38774_at	STX17	8117	syntaxin 17
38925_s_at10006	SH3BP1	10309	spectrin SH3 domain-binding protein 1
39735_at	CPLA3	8629	calcium phospholipid dependent kinase 3
40147_at	VAT1	10498	vesicle amine transport protein 1
41462_at	SFX2	8543	sorting nexin 2

Conclusions

- **There is a fundamental difference in the resting CD4⁺ T cell reservoir in viremic versus aviremic patients. In the former, true latency likely does not exist as cells are continually poised to express virus. In the latter, much greater stability exists and this may represent a truly latent reservoir of virus.**
- **Active viral replication, as manifested by detectable plasma viremia, has a significant impact on the physiologic state of resting CD4⁺ T cells in infected viremic patients, and in turn, allows release of cell-free HIV-1 without exogenous activation stimuli.**

Impact of Viral Replication and Viremia on Lymphocyte Subsets in HIV-Infected Individuals



**CD4+ T Cell
Reservoirs**



B Cells



NK Cells



The
**New England
Journal of Medicine**

Established in 1812 as THE NEW ENGLAND JOURNAL OF MEDICINE AND SURGERY

VOLUME 309

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NUMBER 8

**Abnormalities of B-Cell Activation and
Immunoregulation in Patients with the
Acquired Immunodeficiency Syndrome**

H.C. Lane, H. Masur, L.C. Edgar, G. Whalen,
A.H. Rook, and A.S. Fauci

PNAS

Proceedings of the National Academy of Sciences
of the United States of America

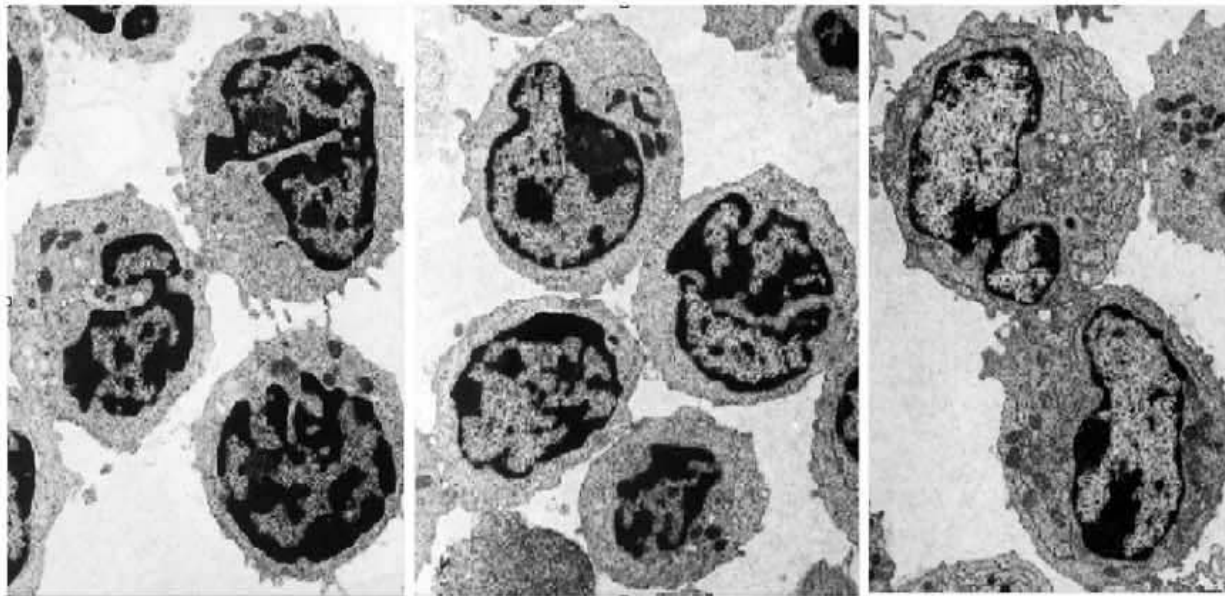
August 28, 2001 | vol 98 | no. 18

HIV-1 Induces Phenotypic and Functional Perturbations of B Cells in Chronically Infected Individuals

Susan Moir, Angela Malaspina, Kisani M. Ogwaro, Eileen T. Donoghue, Claire W. Hallahan, Linda A. Ehler, Shuying Liu, Joseph Adelsberger, Réjean Lapointe, Patrick Hwu, Michael Baseler, Jan M. Orenstein, Tae-Wook Chun, Jo Ann M. Mican, and Anthony S. Fauci

Previous Findings Indicate That HIV-Mediated Hyper-Activation Induces Terminal Differentiation of B Cells

- Loss of CD21 expression
- Reduced proliferation
- Increased immunoglobulin secretion
- Changes in morphology



HIV-negative

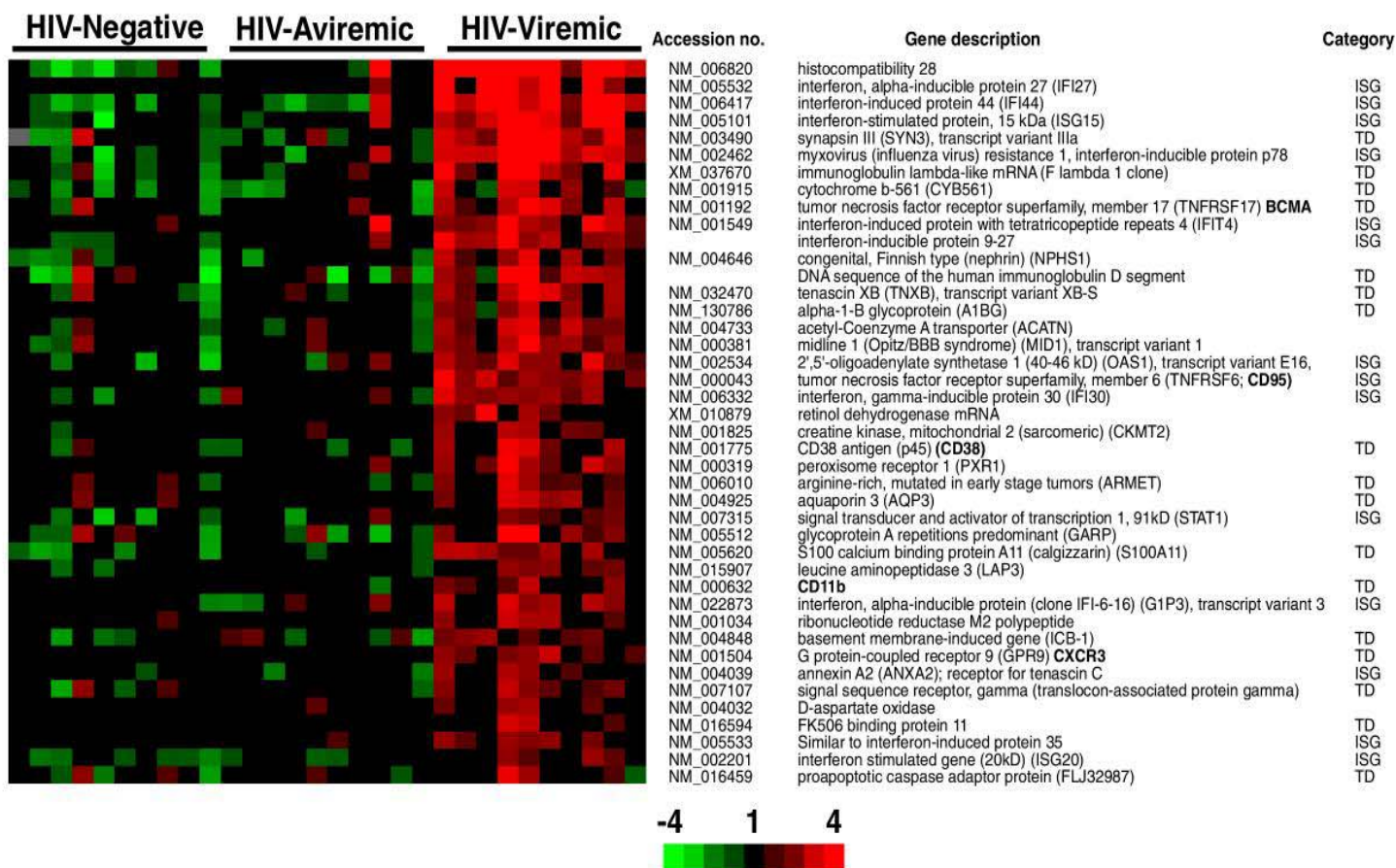
HIV-viremic CD21+

HIV-viremic CD21-

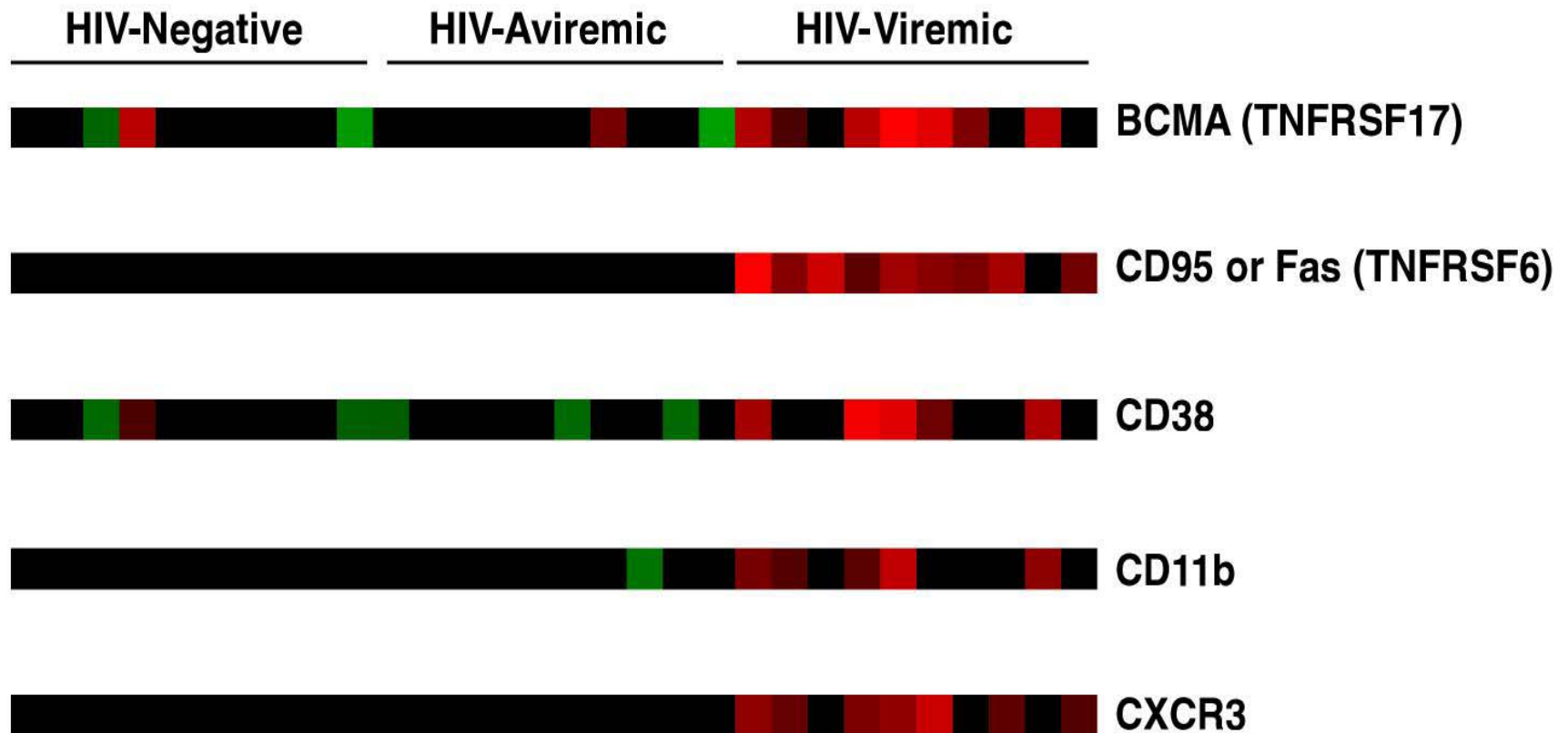
**Determination of B Cell
Gene Expression in
Viremic versus
Aviremic HIV-Infected
versus HIV-Negative
Individuals**

DNA Microarray Analysis: Genes Upregulated in B Cells of HIV-Viremic Patients

- Major categories of genes upregulated:
 - Interferon-stimulated genes
 - Genes associated with B cell terminal differentiation

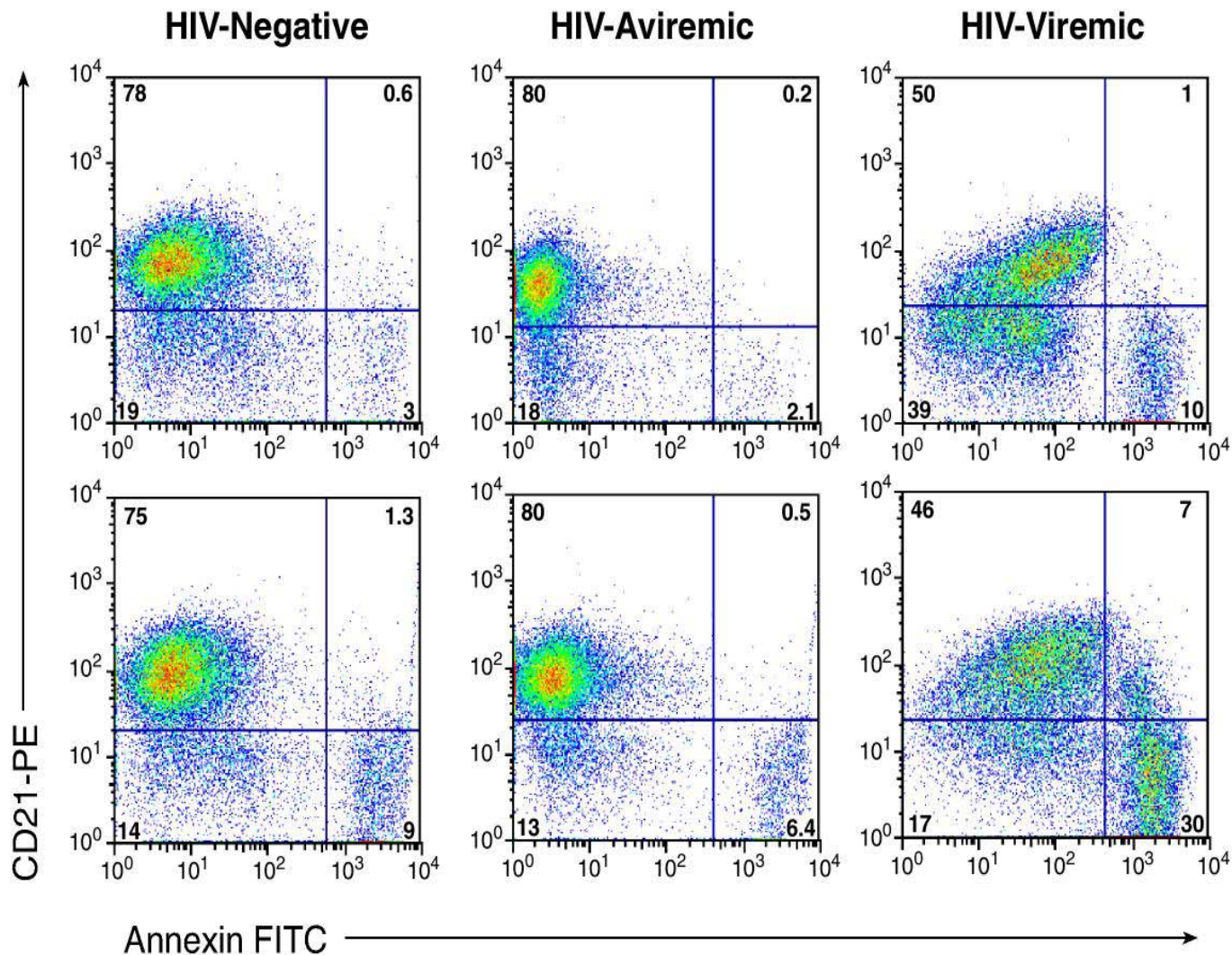


Microarray Sub-analysis: Genes of Cell Surface Receptors Associated with Interferon Induction and B Cell Terminal Differentiation



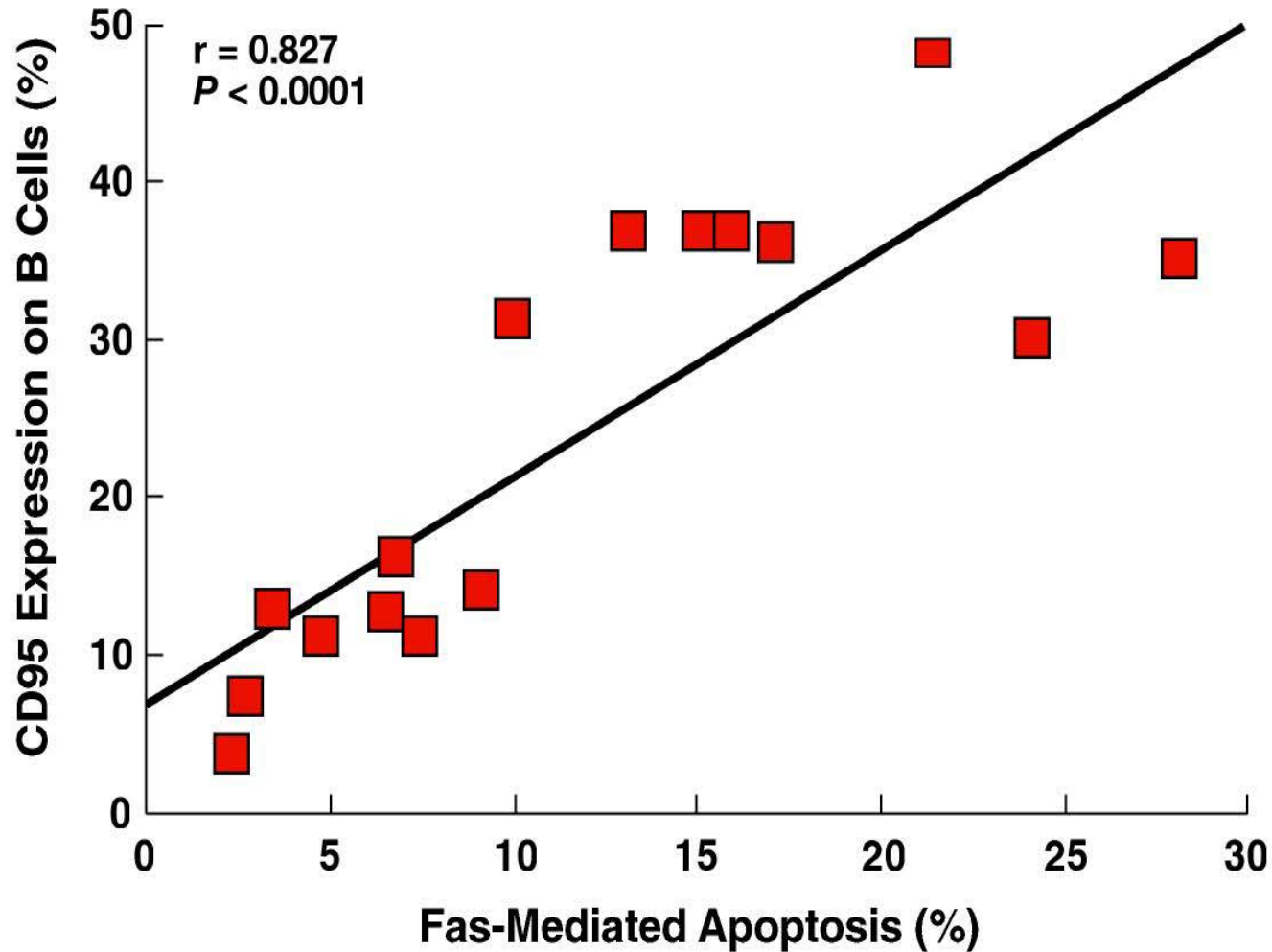
Increased Susceptibility of B Cells of HIV-Viremic Patients to Fas-Mediated Apoptosis

**B Cells
Untreated**

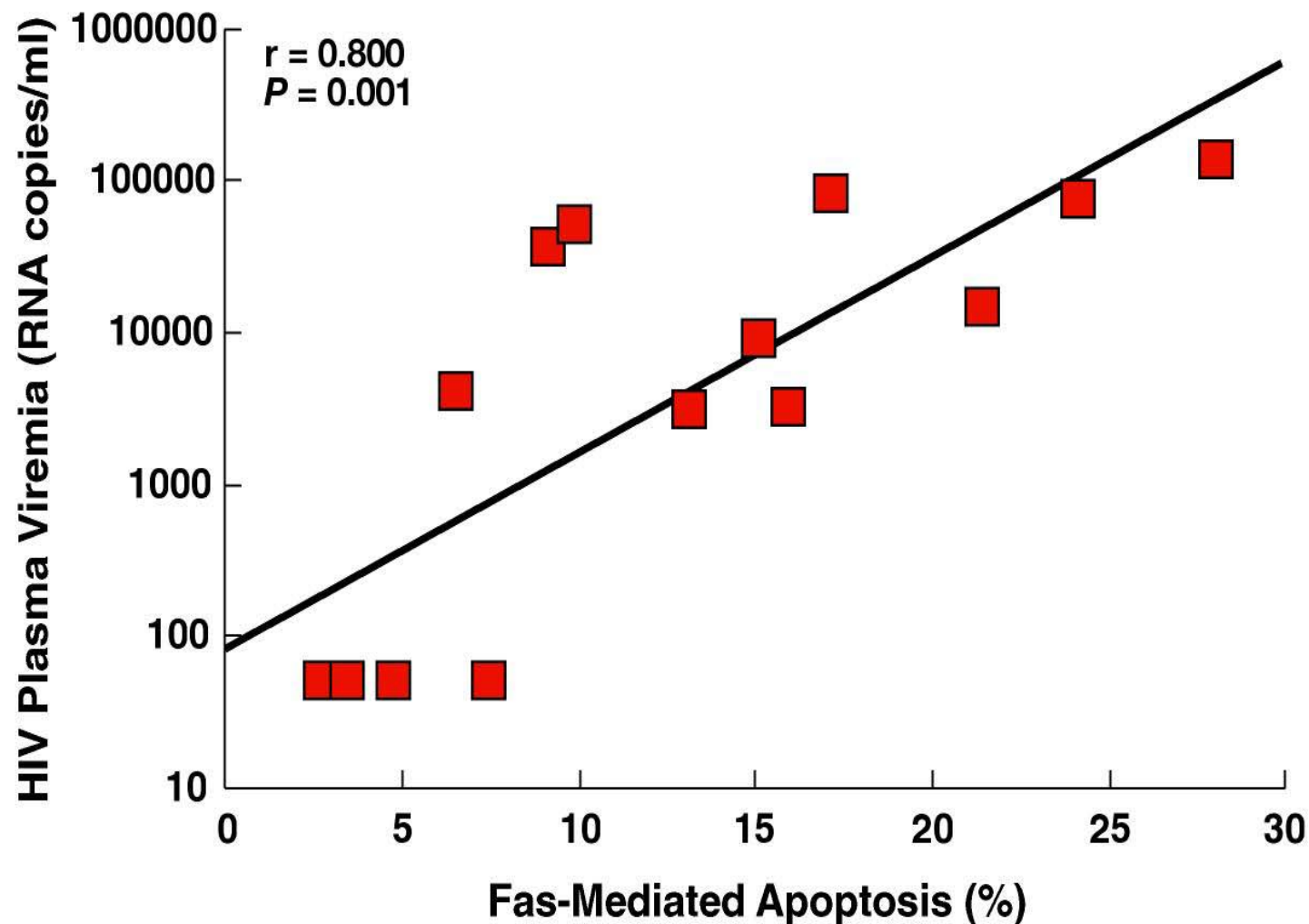


**B Cells
Treated
With
FasL**

Direct Correlation Between Level of Fas Expression on B Cells and Susceptibility of B Cells to Fas-Mediated Apoptosis



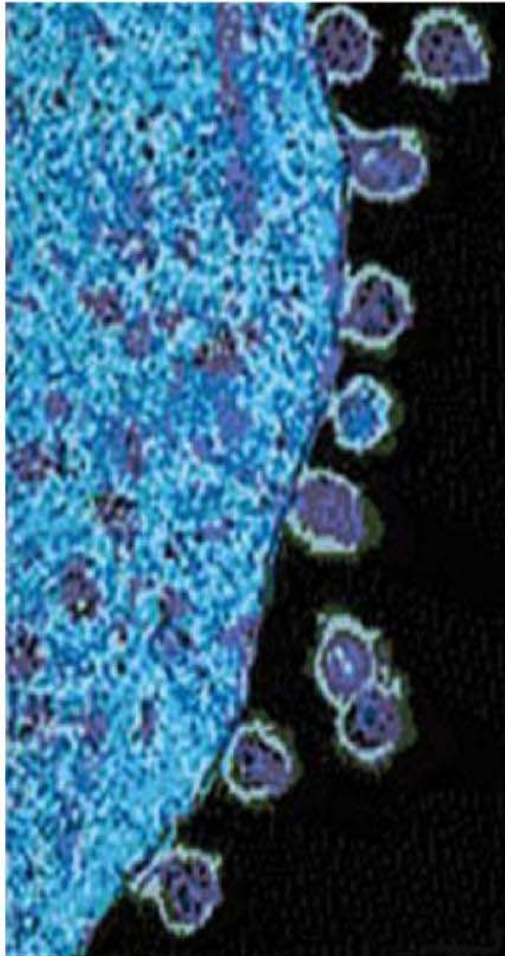
Direct Correlation Between HIV Plasma Viremia and Susceptibility of B Cells to Fas-Mediated Apoptosis



Summary

- **A large number (~42) of B cell genes are upregulated in HIV-viremic vs HIV-aviremic and HIV-negative individuals; 75% are interferon-stimulated or associated with B cell terminal differentiation.**
- **Changes in expression of TNFSF receptors such as CD95 (Fas) are associated with increased B cell death by apoptosis.**

Impact of Viral Replication and Viremia on Lymphocyte Subsets in HIV-Infected Individuals



**CD4+ T Cell
Reservoirs**

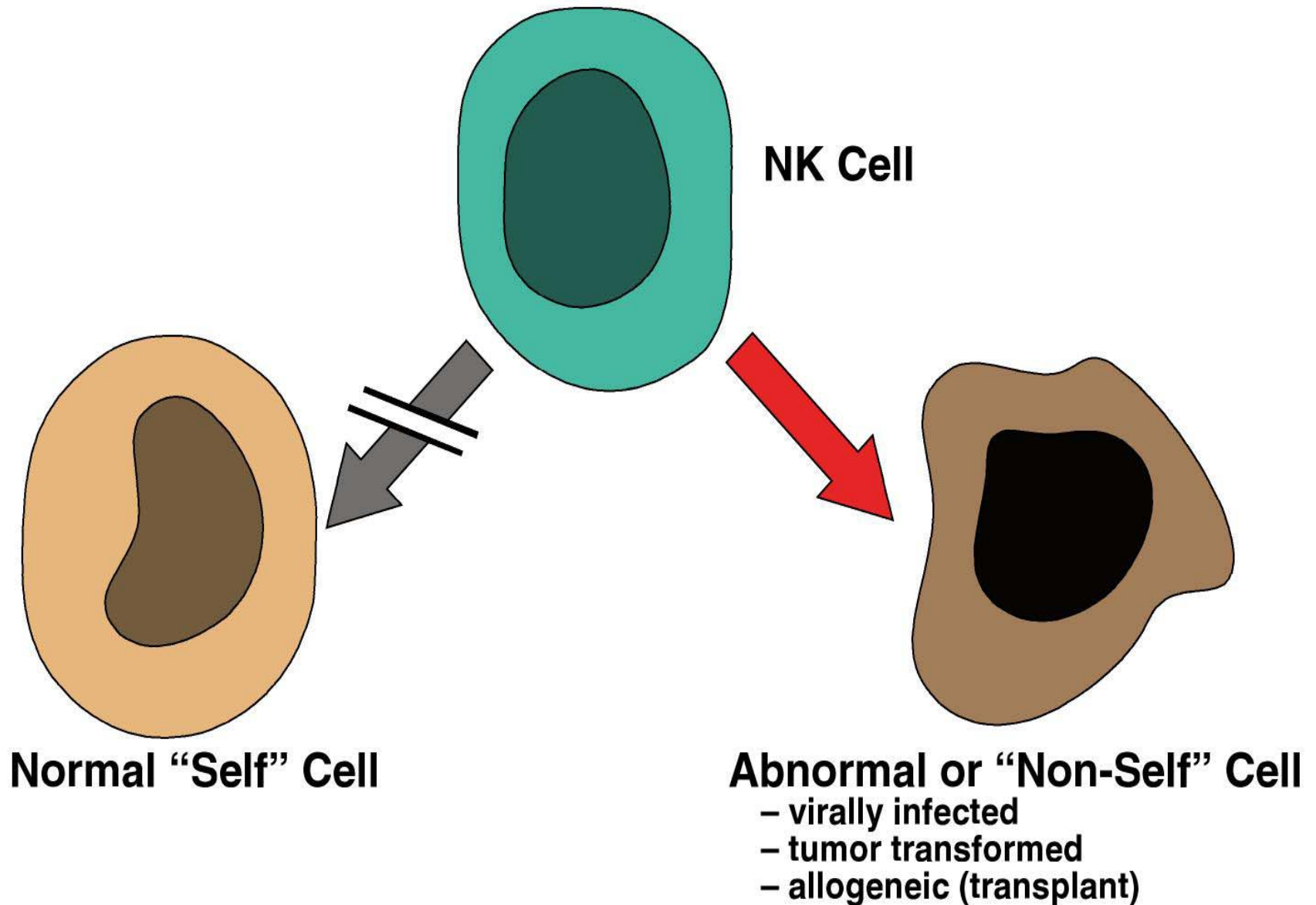


B Cells



NK Cells

Function of NK Cells



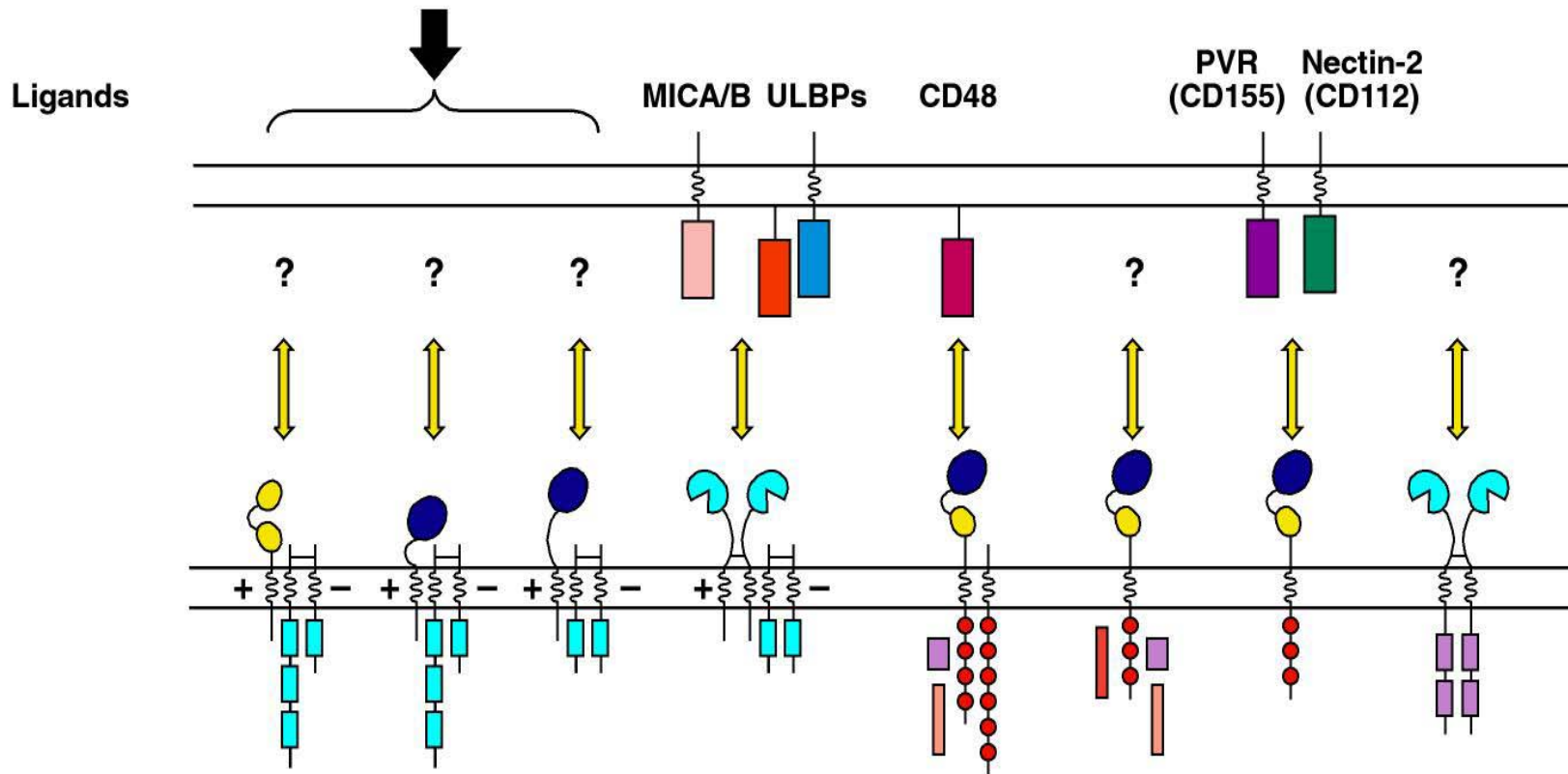
2 Major Types of NK Cell Receptors

■ Activating

■ Inhibitory (Dominant)

NK Cell Activating Receptors

NK Cell Natural Cytotoxicity Receptors



Receptors NKp46 NKp30 NKp44 NKG2D 2B4 NTB-A DNAM-1 NKp80

(Signaling polypeptides)

(ζ/γ)

(ζ/γ)

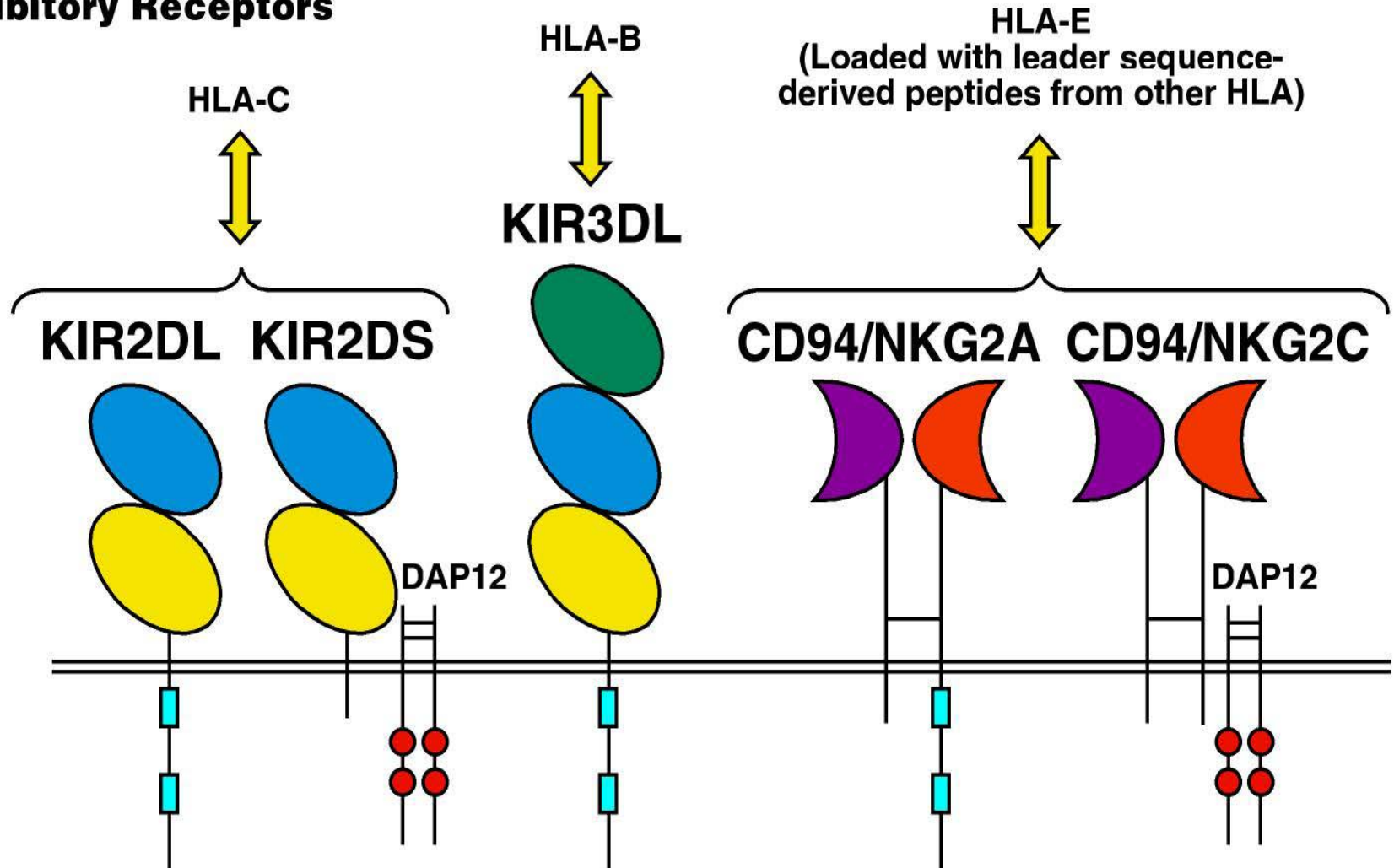
(DAP12)

(DAP10)

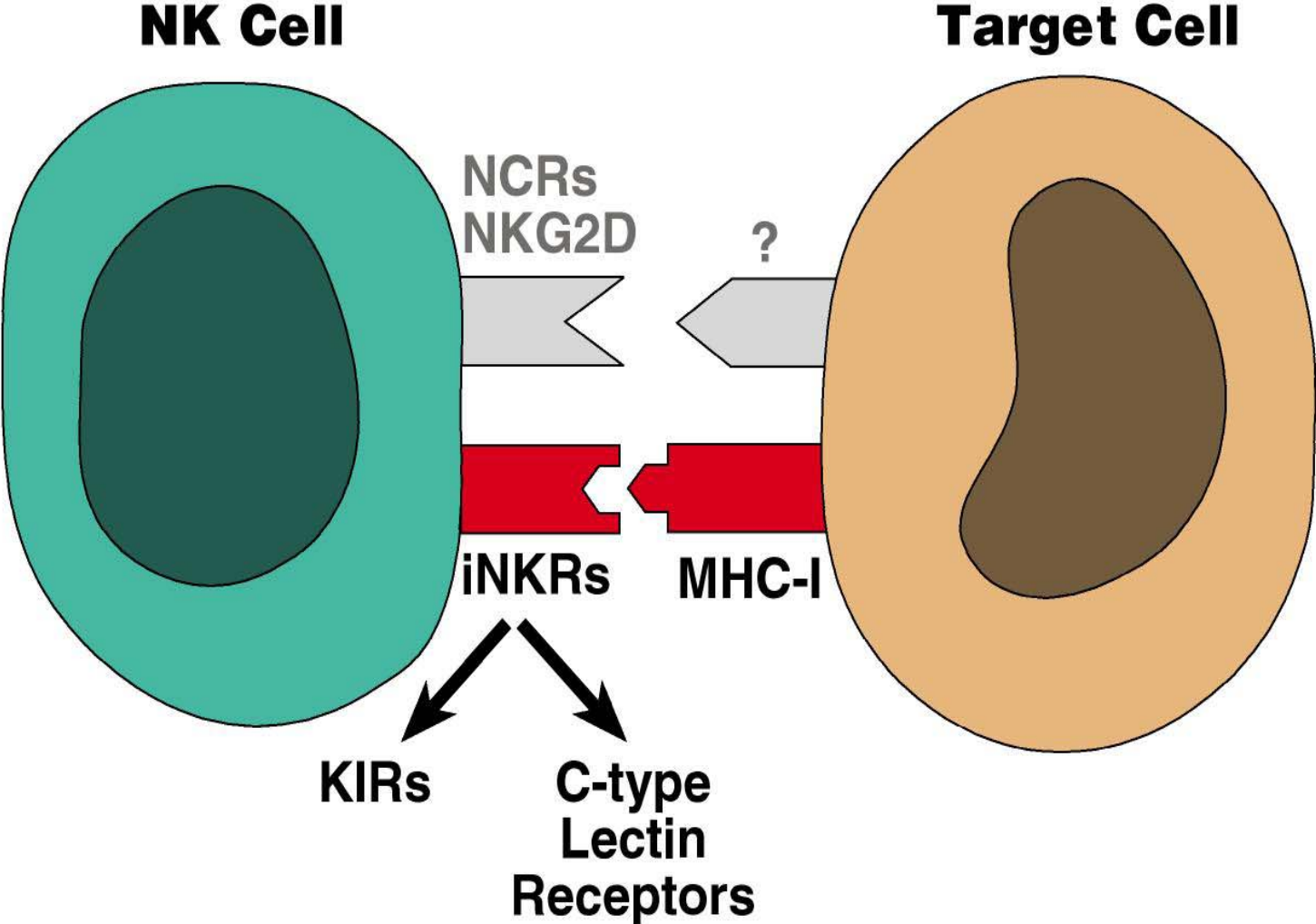
(LAT)

NK Cell Inhibitory Receptors

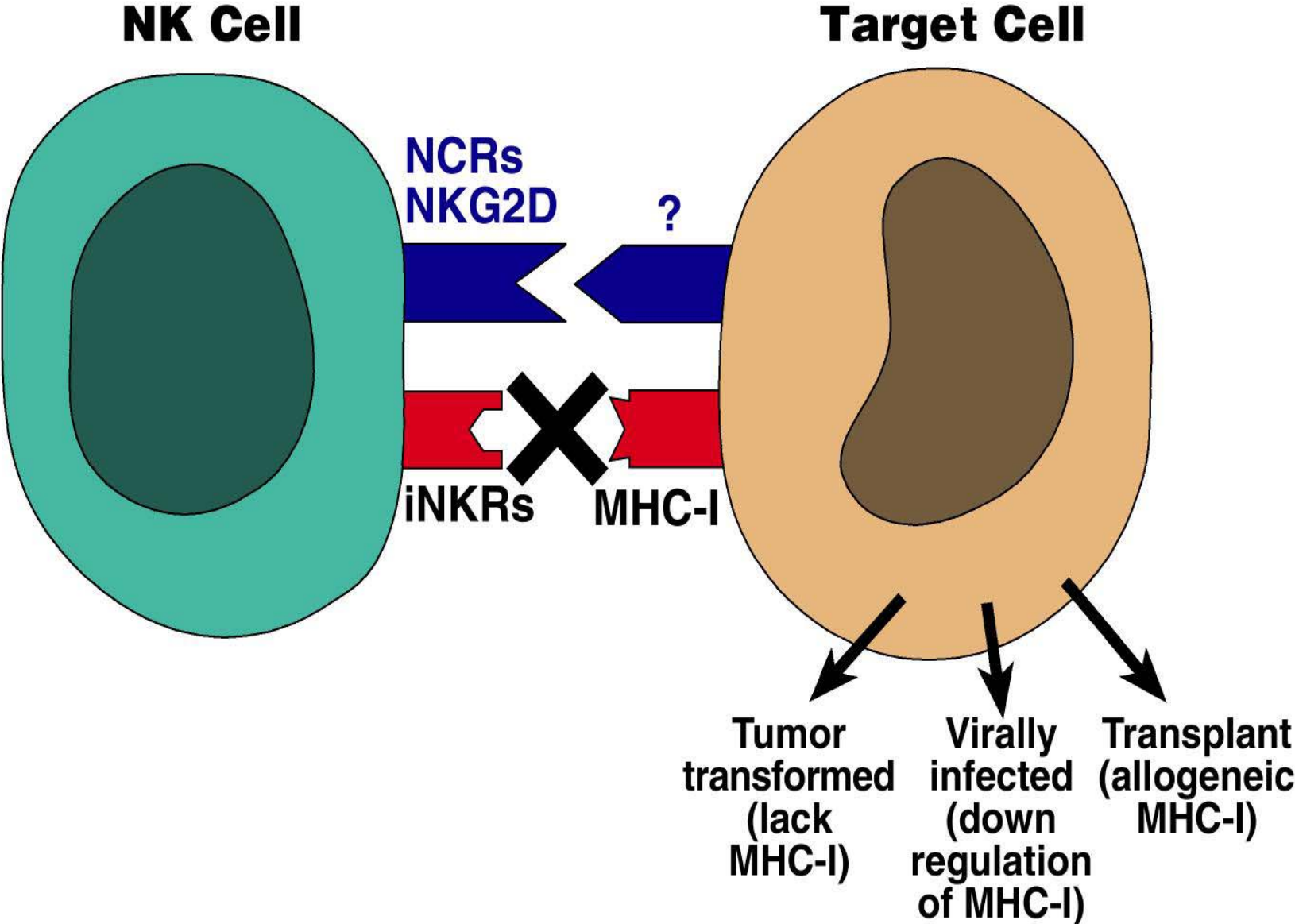
MHC-I Specific Inhibitory Receptors



NK Cell Function



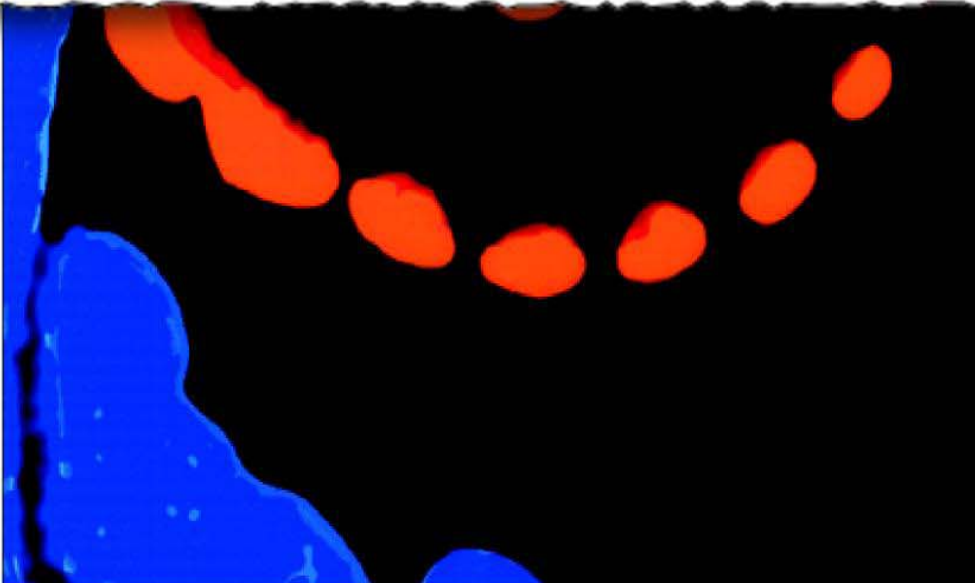
NK Cell Function



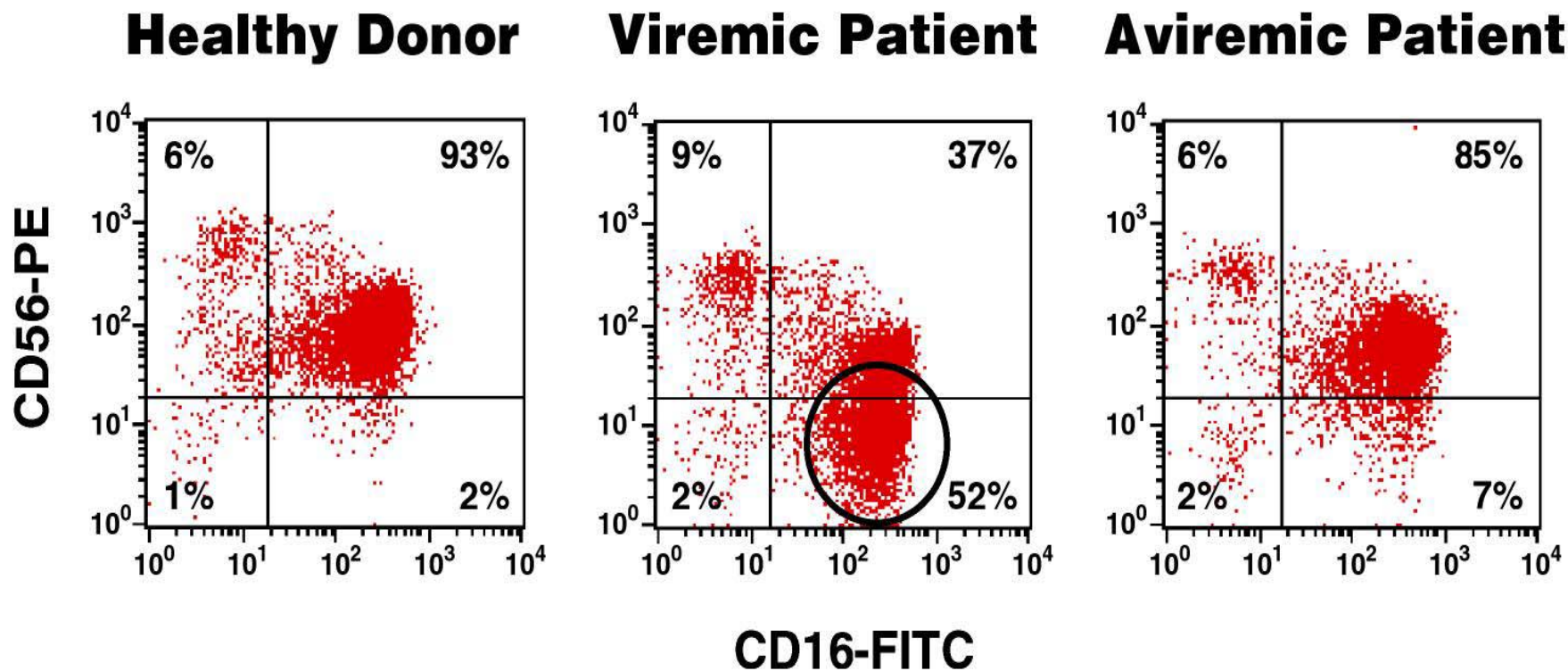
Natural Killer Cells in HIV-1 Infection: Dichotomous Effects of Viremia on Inhibitory and Activating Receptors and Their Functional Correlates

Domenico Mavilio et al

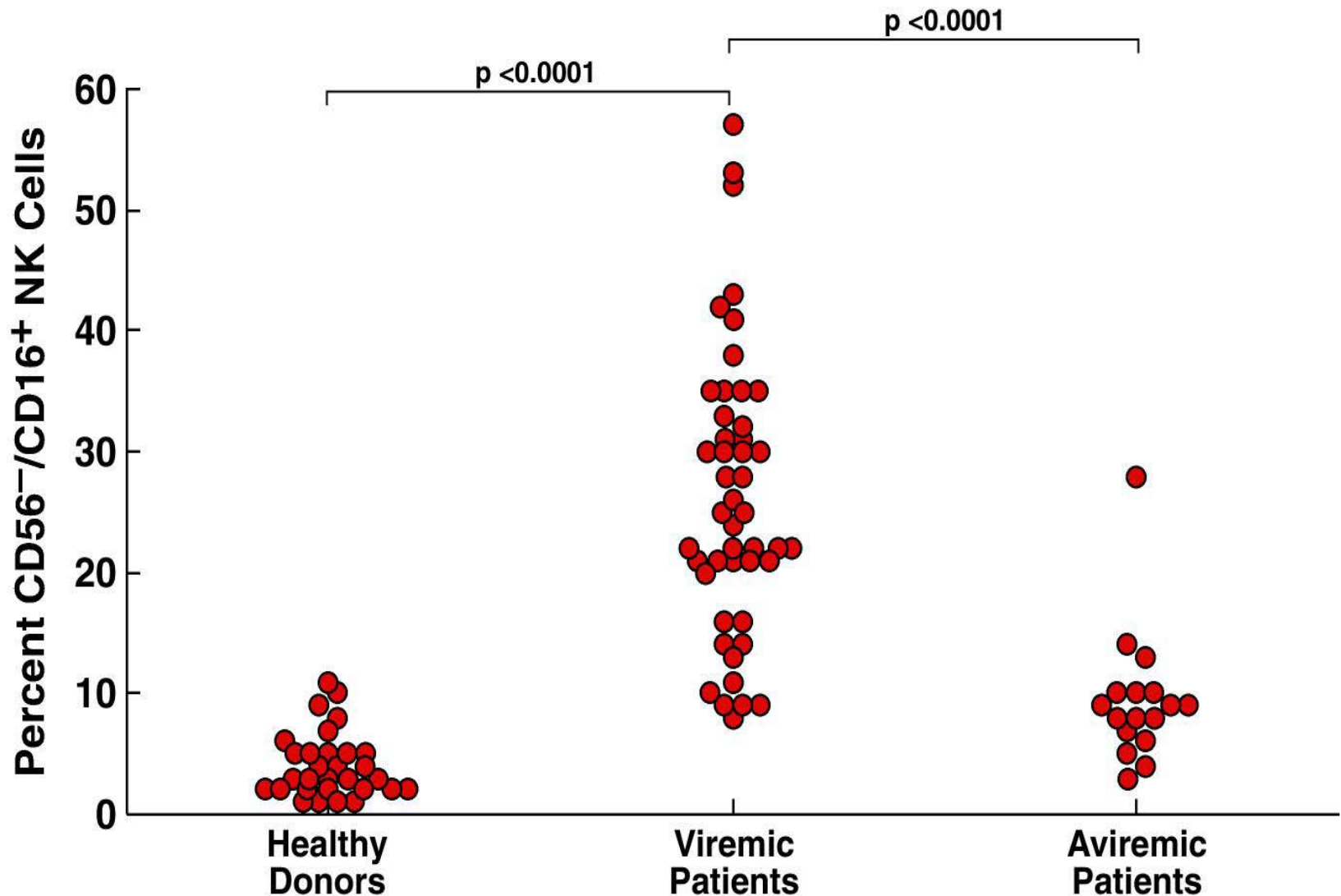
- HIV viremic patients manifest:
 - Decrease in activating receptors (NCRs)
 - Normal or increase in inhibitory receptors



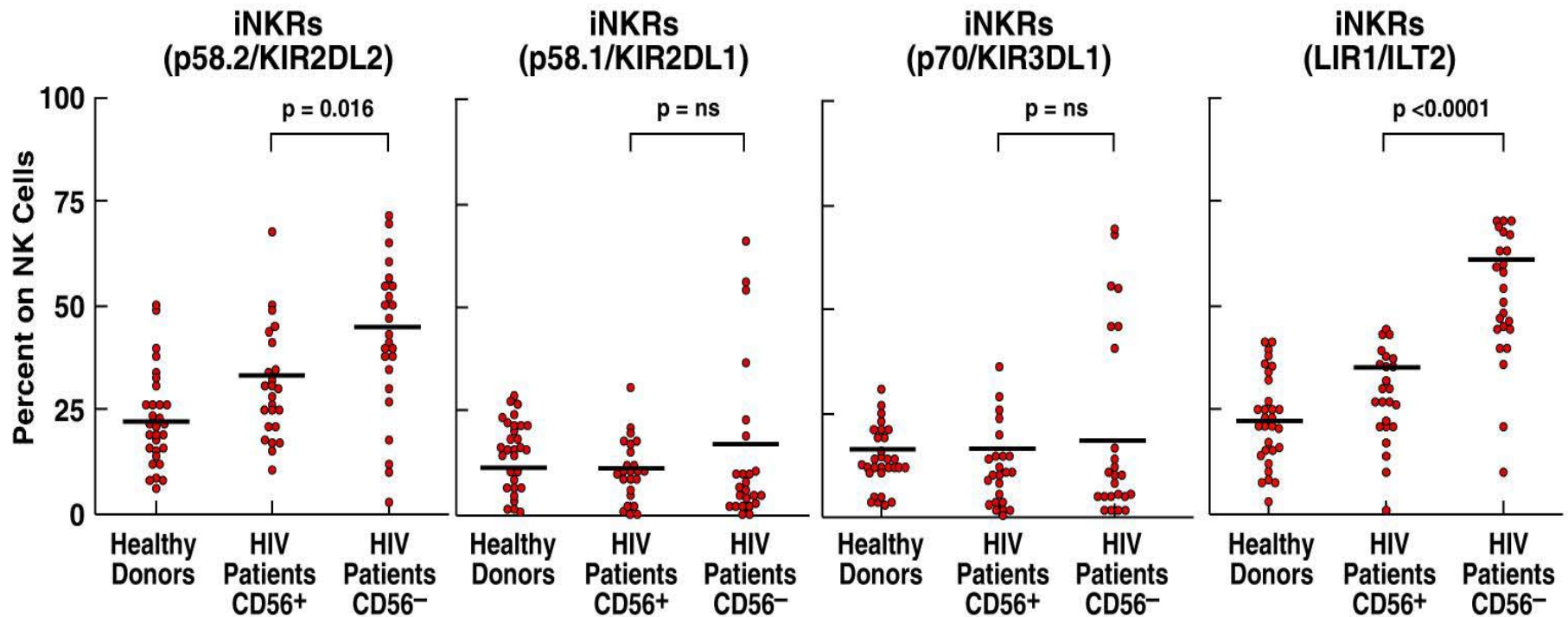
Enrichment of CD56⁻/CD16⁺ NK Cells in HIV-1 Viremic Patients



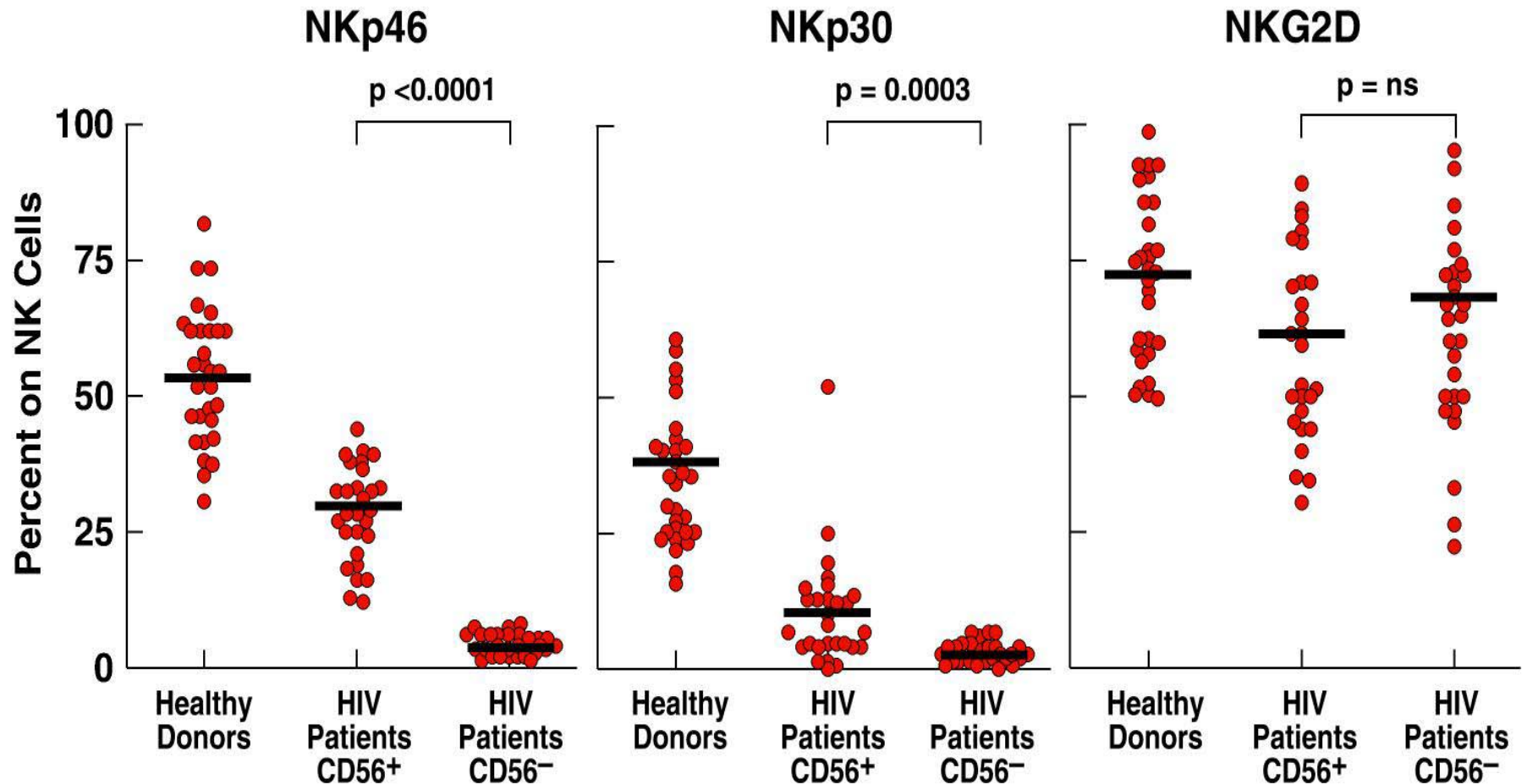
The CD56⁻/CD16⁺ NK Cell Subset is Enriched in HIV-Viremic Patients



Inhibitory NK Receptors are Either Conserved or Increased in CD56⁻ NK Cells of HIV-Viremic Patients



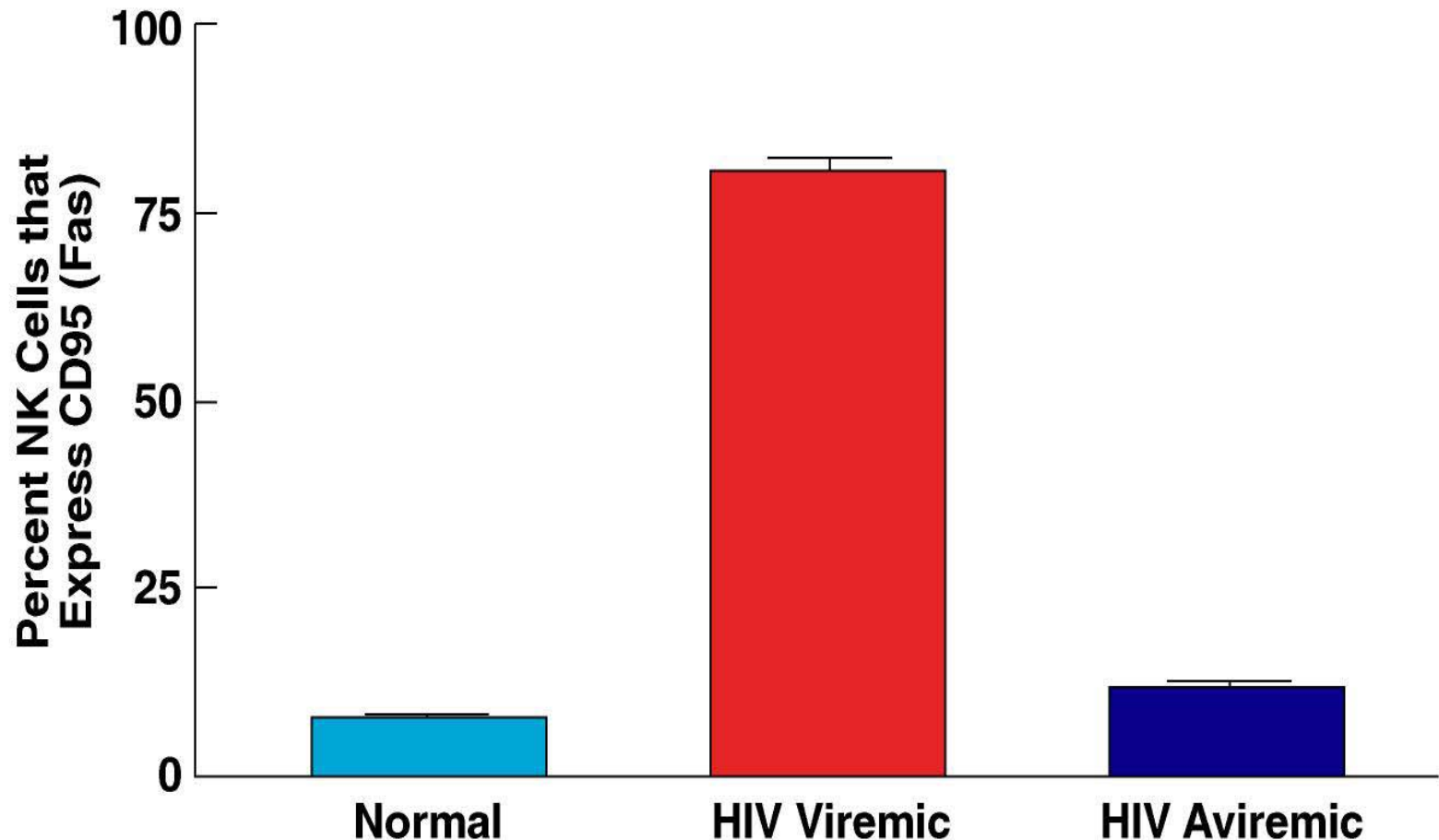
Activating NK Receptors are Decreased in CD56⁻ NK Cells of HIV-Viremic Patients



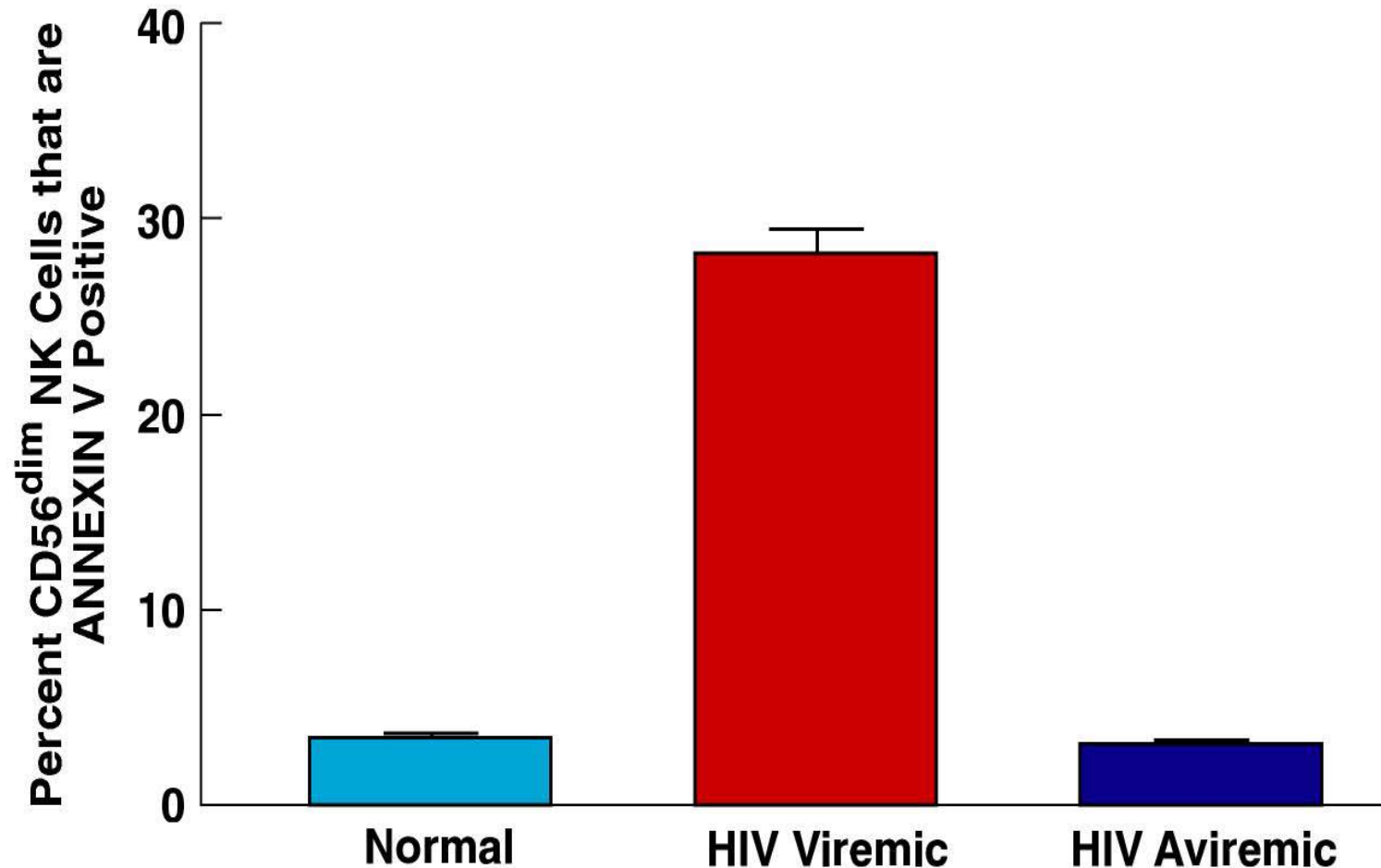
Effect of HIV Viremia on CD56-NK Cells

- Decreased production of perforin and granzyme
- Decreased secretion of TNF- α and interferon- γ

NK Cells from HIV Viremic Individuals Express Fas on their Surface at a Significantly Higher Level than do NK Cells from Normal and HIV-Aviremic Individuals



NK Cells from HIV-Viremic Individuals Undergo Increased Apoptosis upon Exposure to sFasL *in vitro*

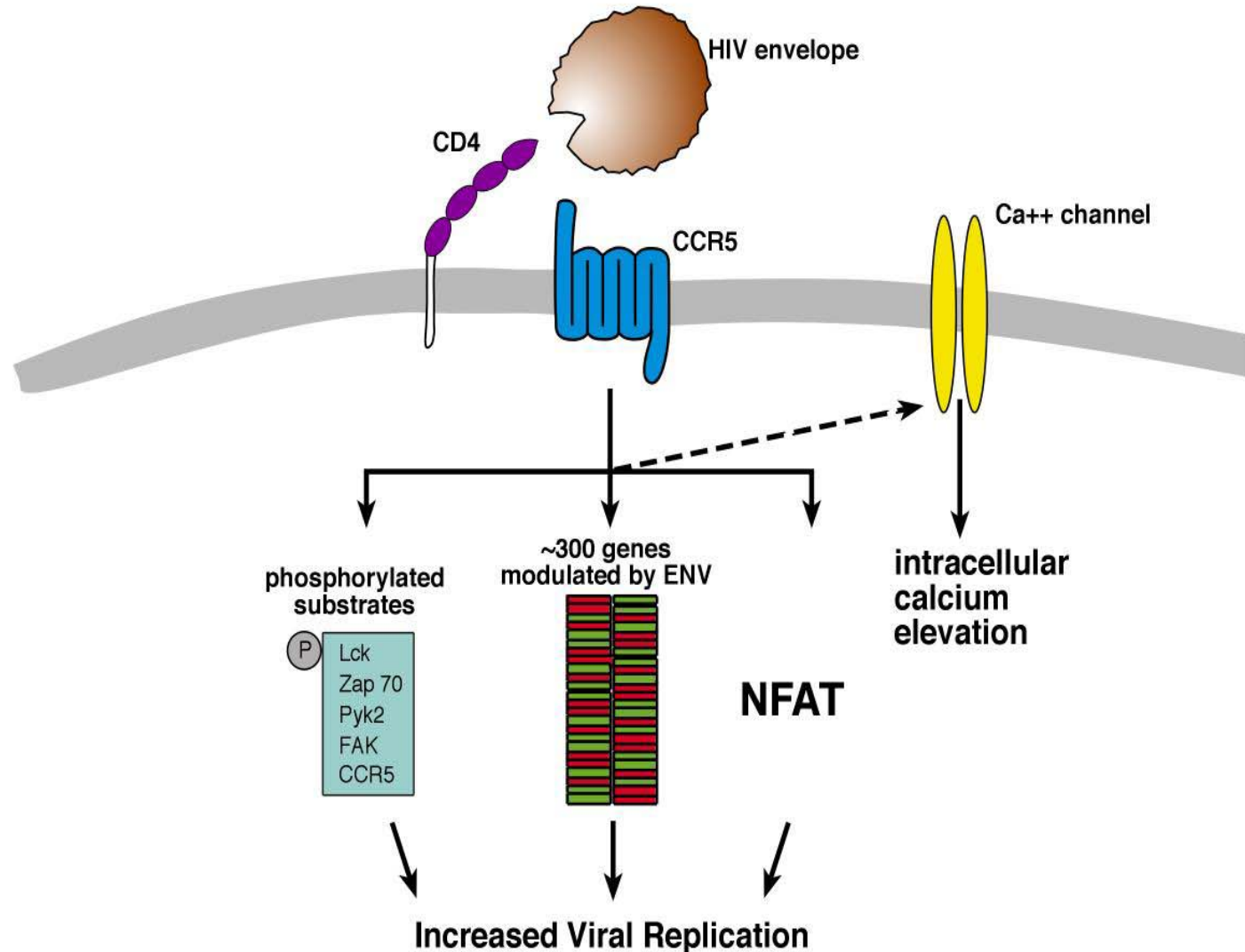


Effect of HIV Viremia on NK Cell Phenotype and Function

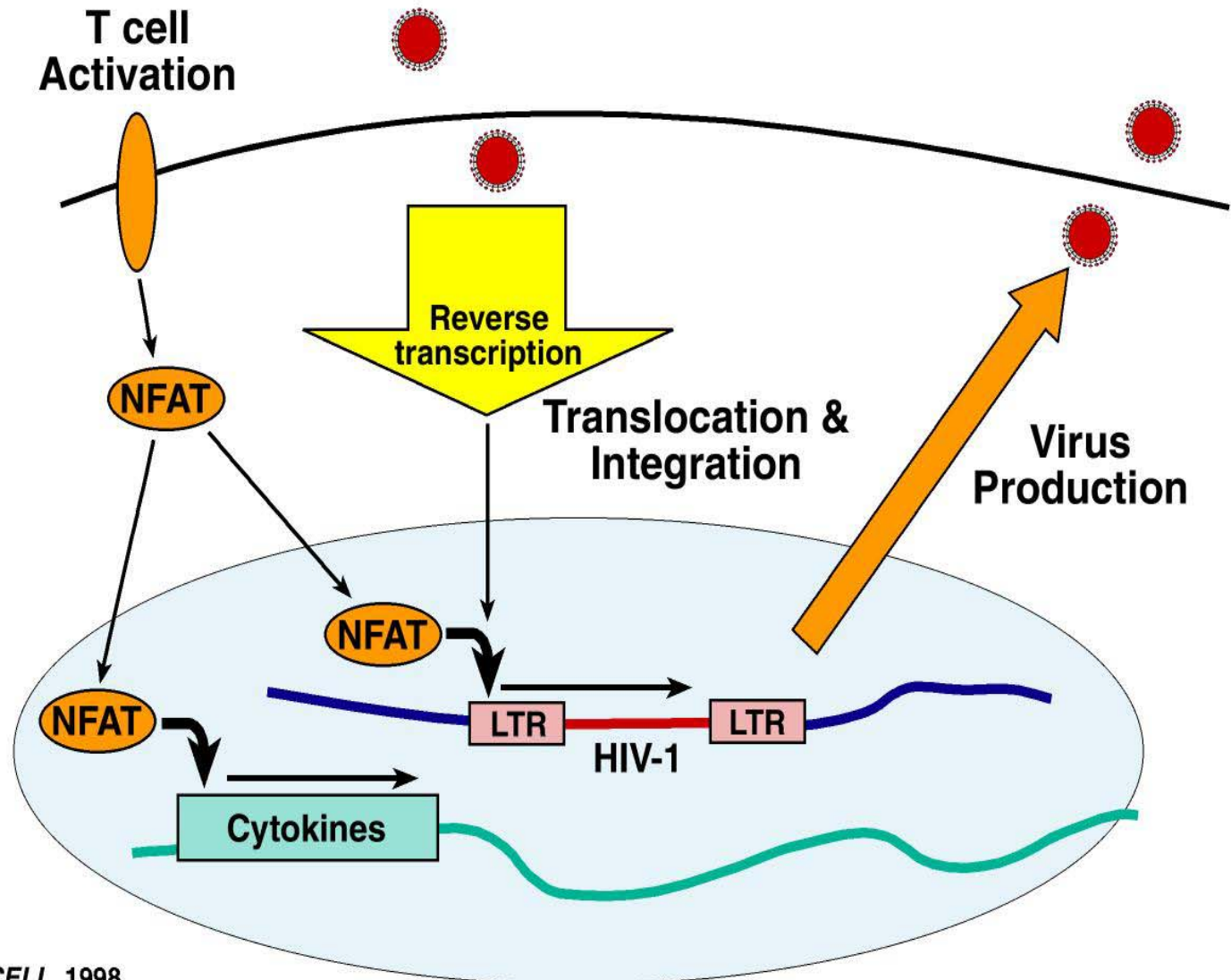
- HIV-viremic patients manifest a dichotomy of expression of NK cell receptors: inhibitory receptors are maintained or increased while activating receptors are decreased.
- This dichotomy is reflected in decreased cytotoxic function as well as a decrease in production of perforin and granzyme and a decrease in secretion of TNF- α and IFN- γ .
- Viremia is associated with an enrichment of CD56⁻/CD16⁺ subset of NK cells. The defects in receptor phenotype and function are concentrated within this abnormal subset.
- NK cells from viremic patients express increased levels of Fas on their surface and the CD56^{dim}/CD16⁺ subset responds to sFasL with increased apoptosis.

Direct Effects of HIV Envelope on Immune Cell Function

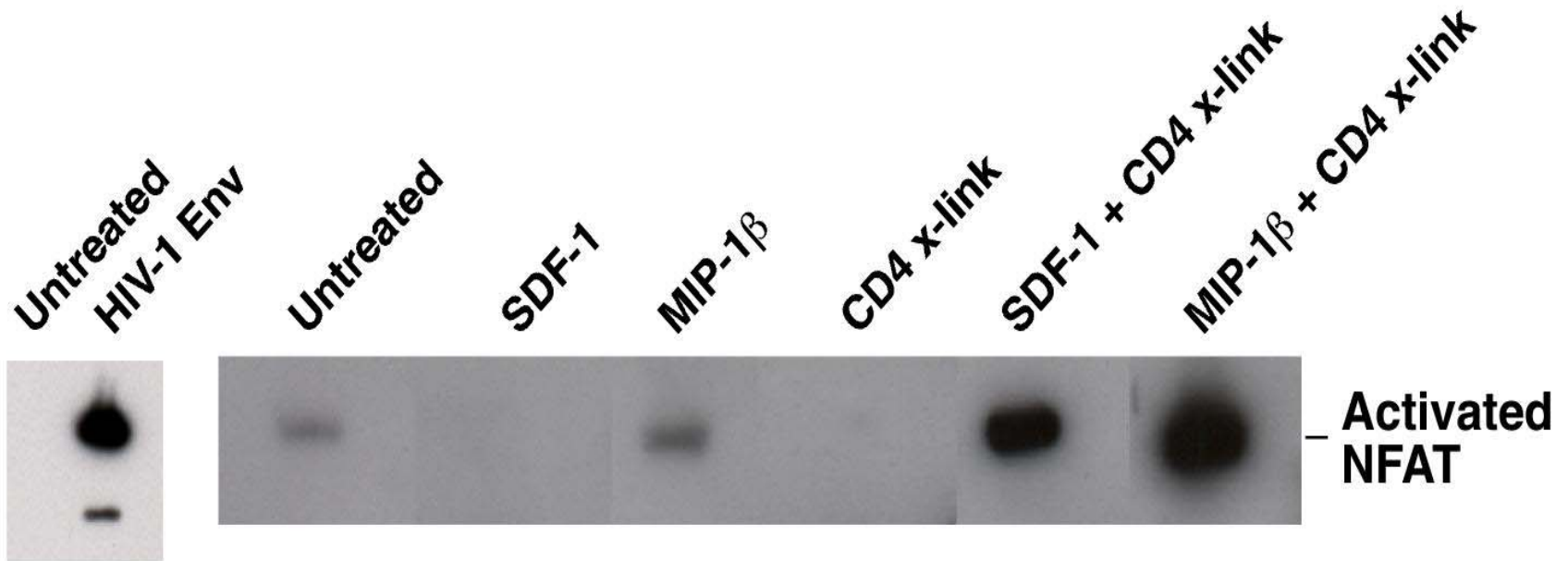
Role of HIV Envelope in the Induction of Aberrant Signal Transduction



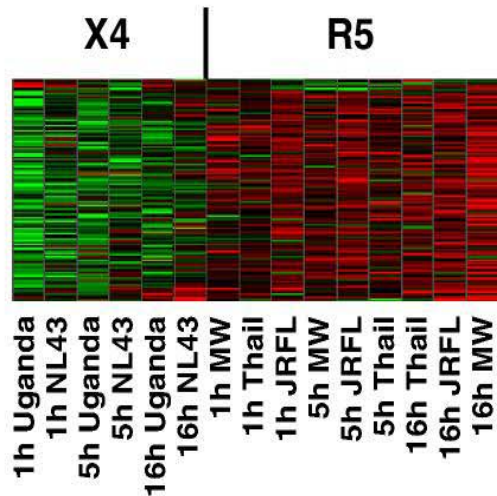
The Role of NFAT in HIV Life Cycle



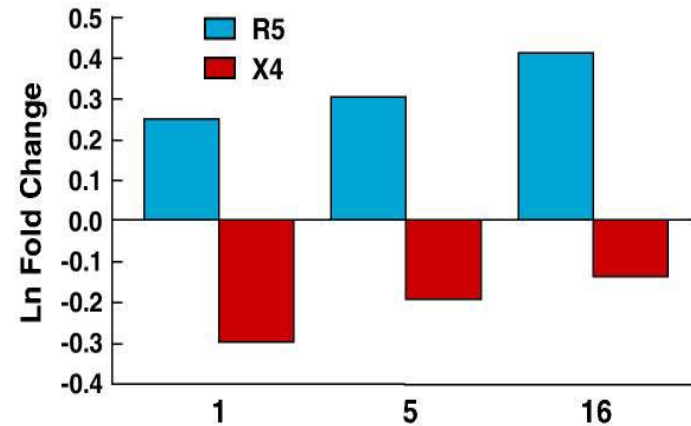
NFAT Activation through HIV Envelope Requires Coordinate Signaling through CD4 and Coreceptor



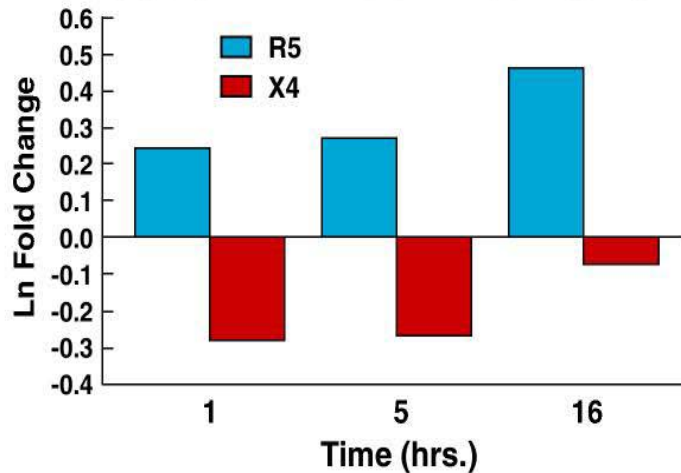
Categories of Genes Differentially Modulated by R5 vs X4 Envelopes



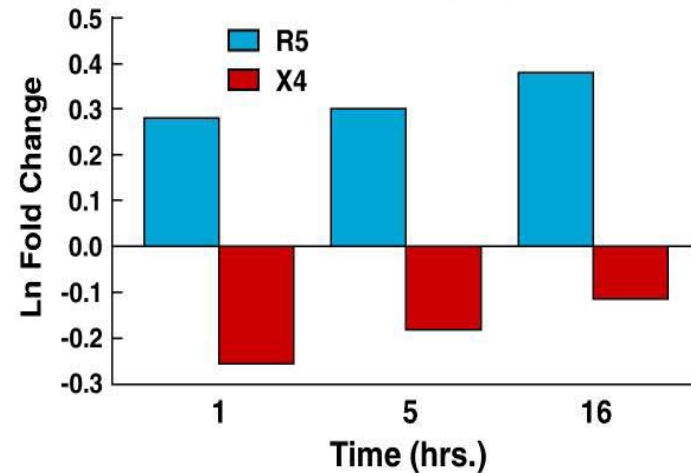
Cluster 4 - "Transcription Factors"



Cluster 4 - "Protein Modification"



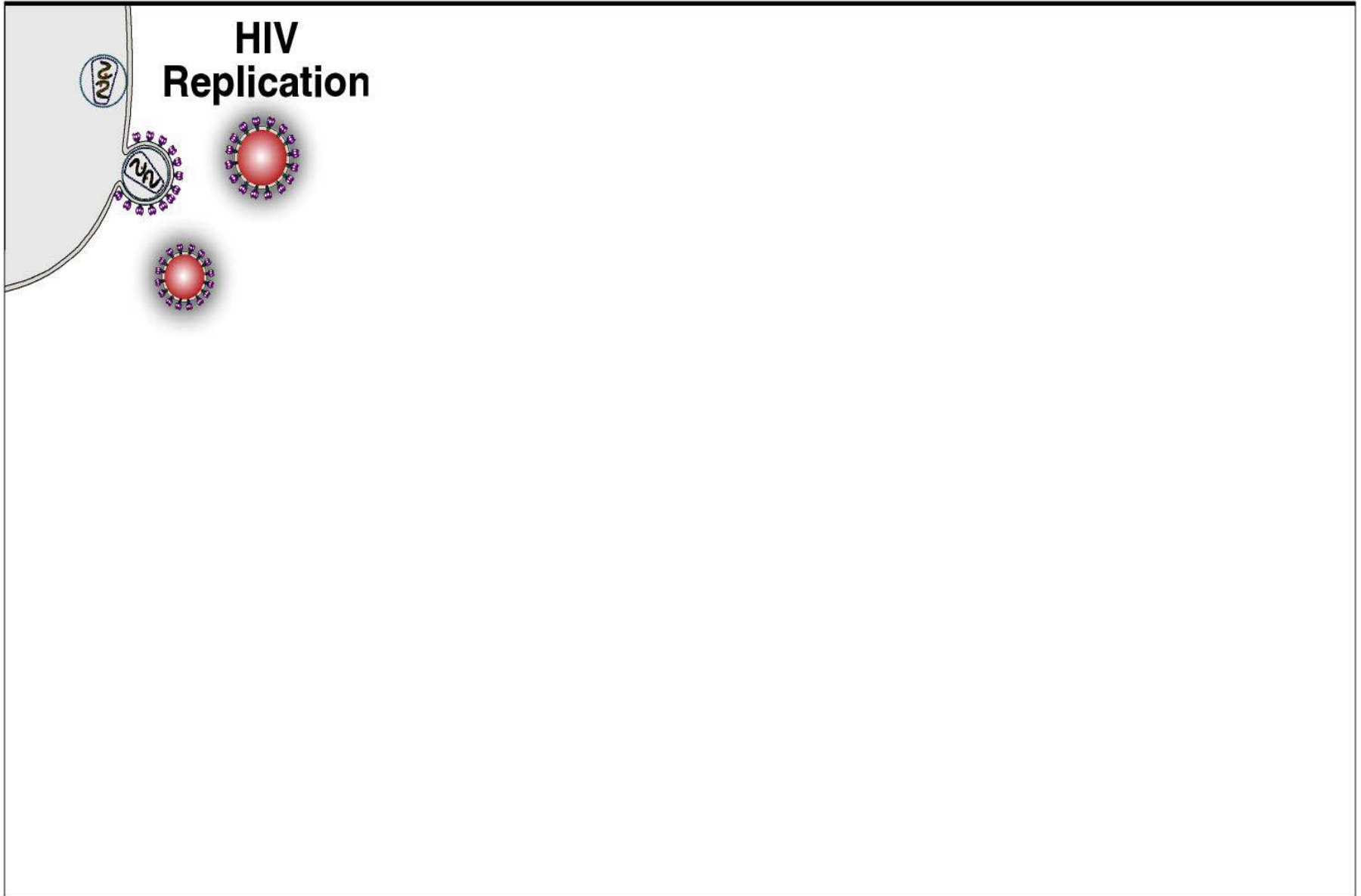
Cluster 4 - "Cell Cycle"



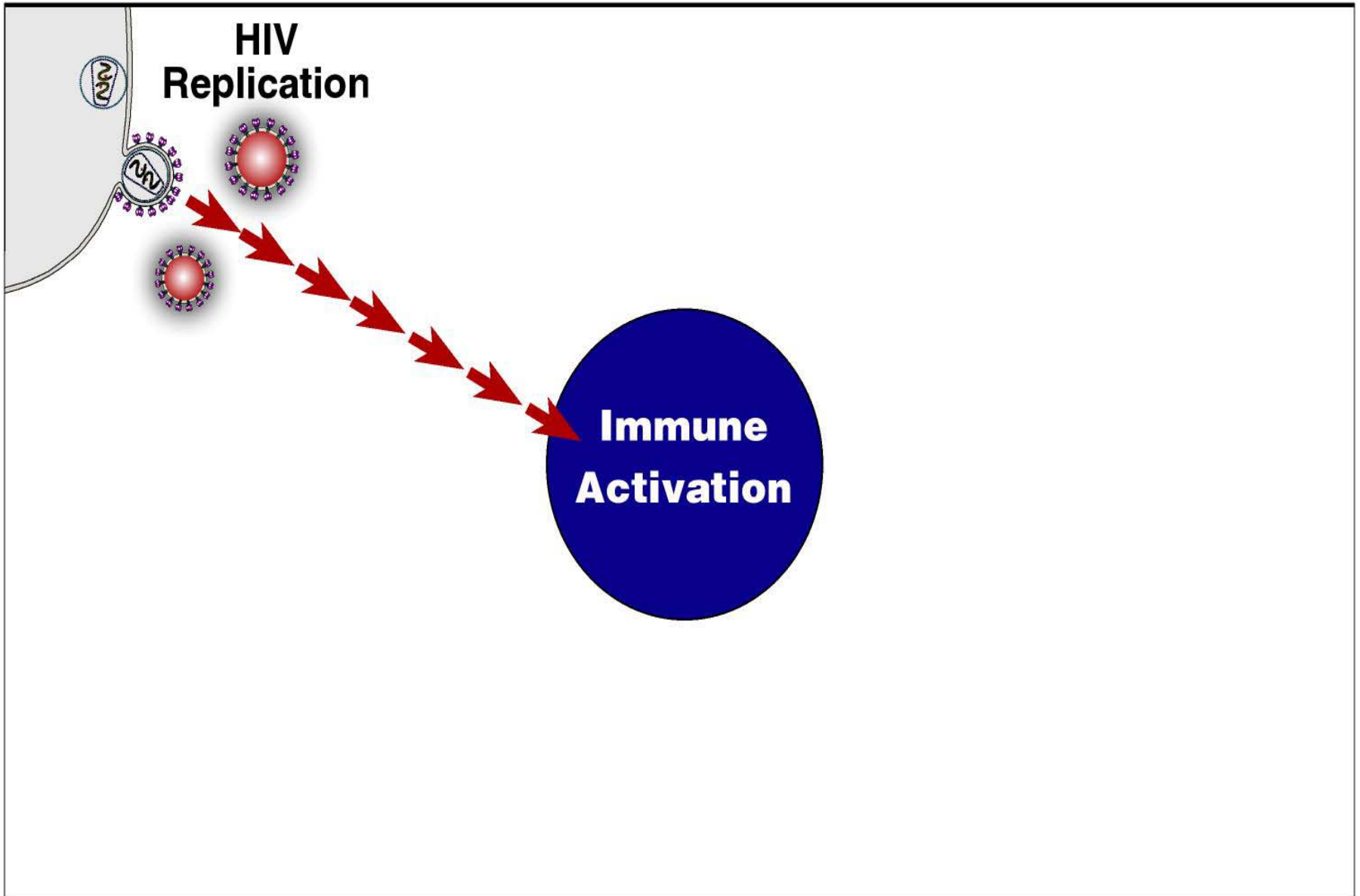
Conclusions

- **HIV envelope modulates PBMC gene expression that is associated with virus replication and the secretion of cytokines involved in immune activation.**
- **This induction of gene expression requires coordinate signaling through CD4 and the respective co-receptor.**
- **Envelopes derived from X4 versus R5 viruses can differentially induce the expression of genes associated with transcription factors, cell cycle, and protein modification.**
- **Thus, HIV envelope-induced cell signaling may play an important role in the propagation of virus replication and the state of aberrant immune activation.**

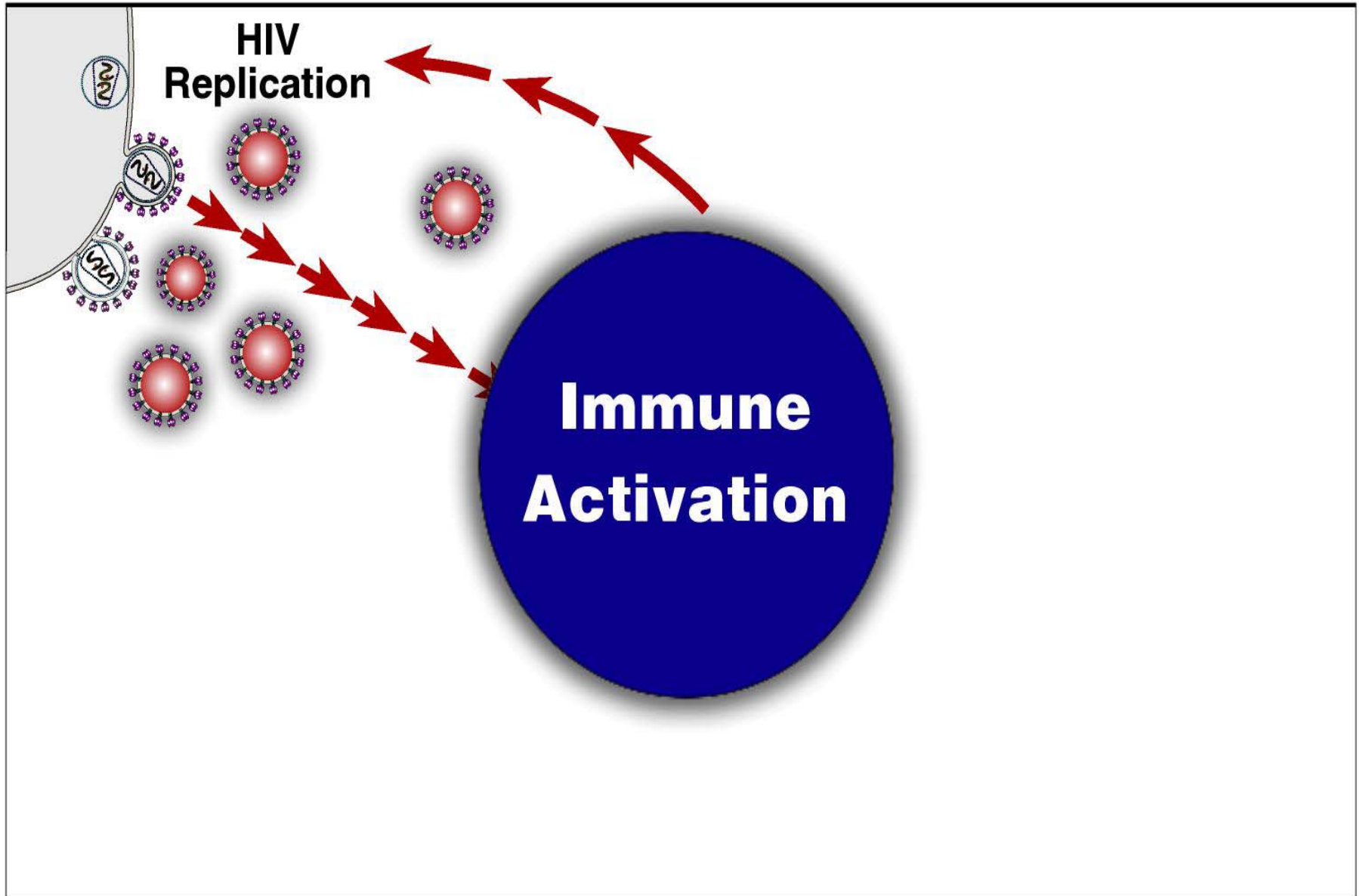
Pathogenic Mechanisms of HIV Disease



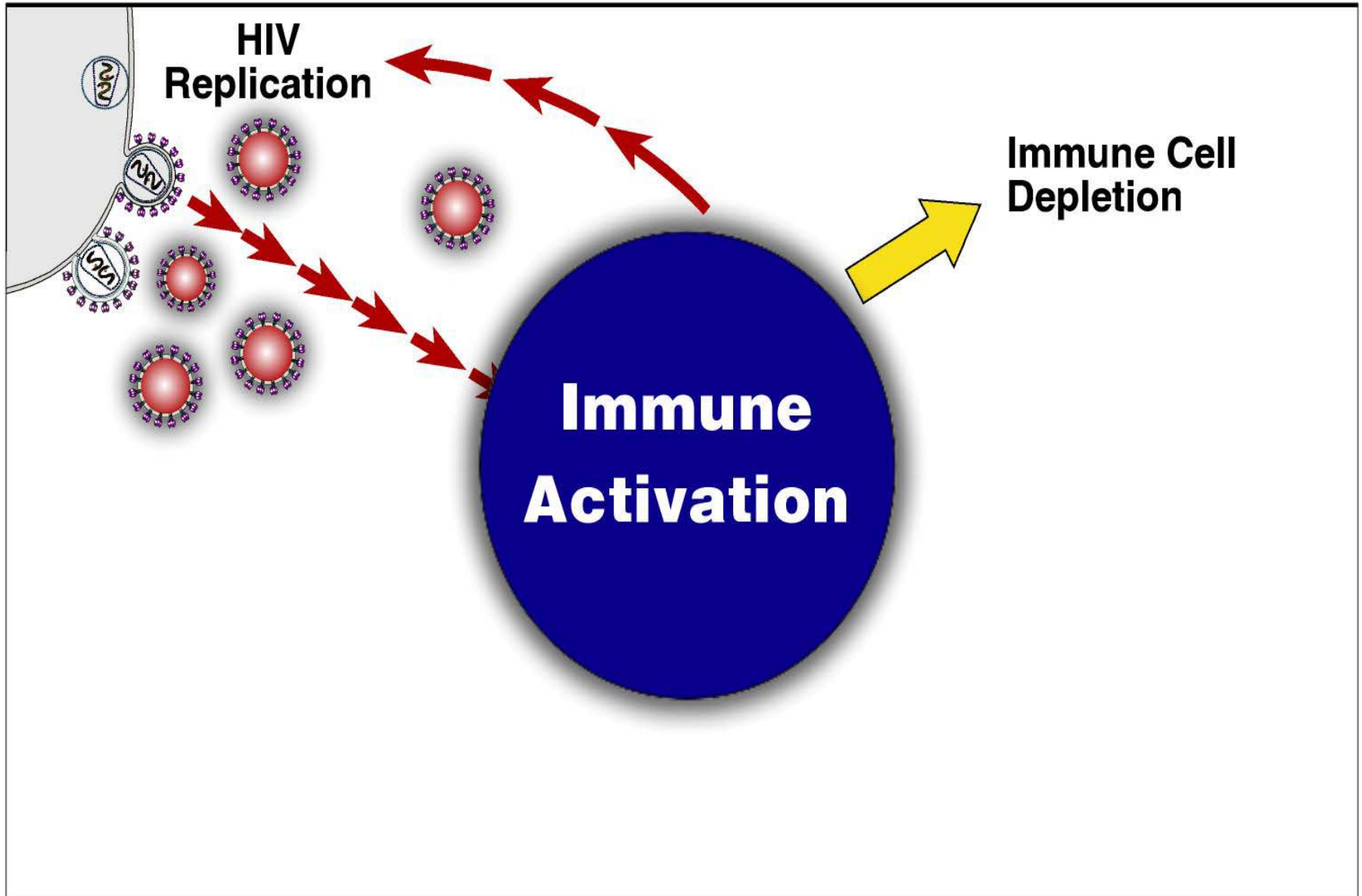
Pathogenic Mechanisms of HIV Disease



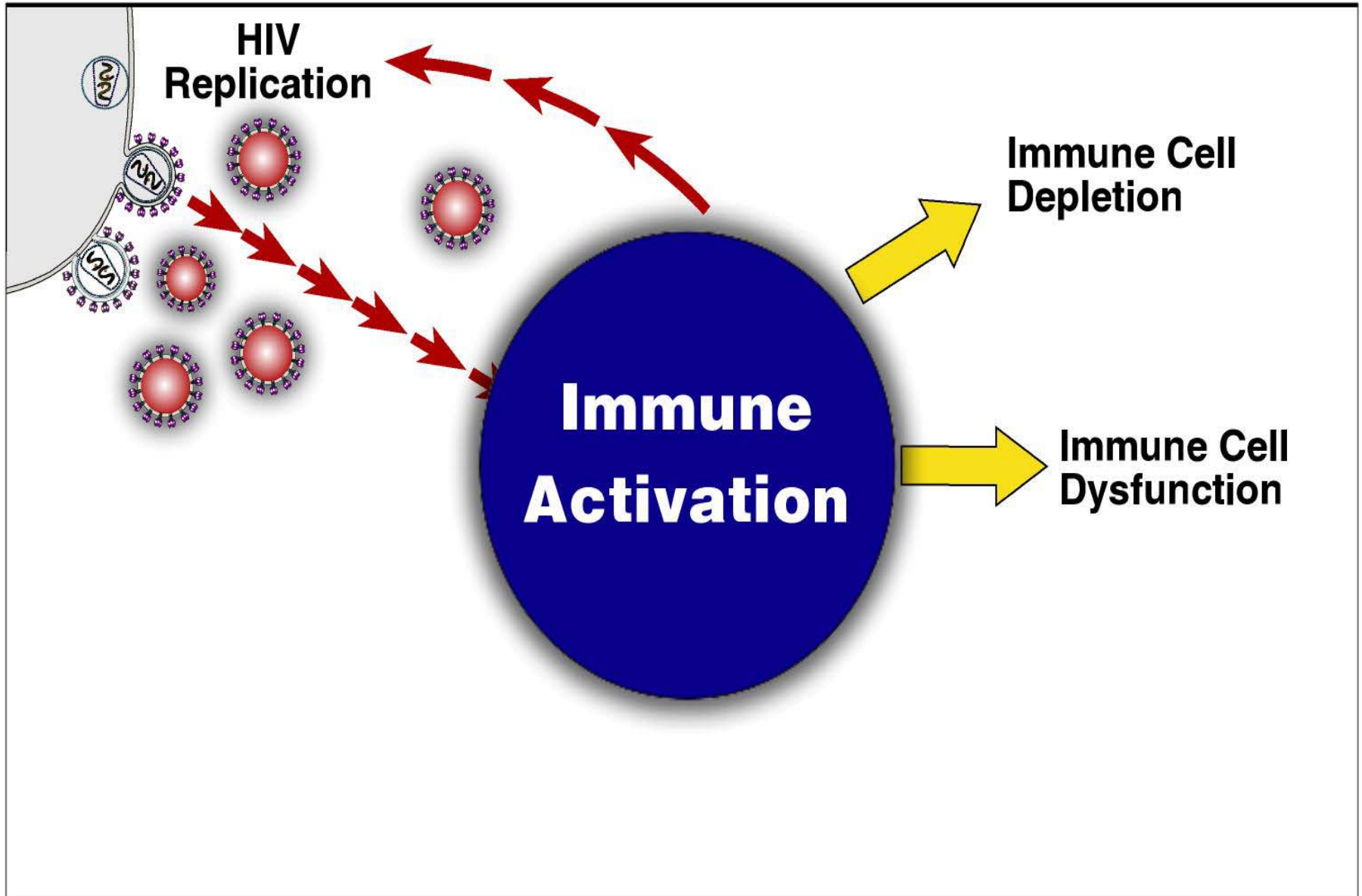
Pathogenic Mechanisms of HIV Disease



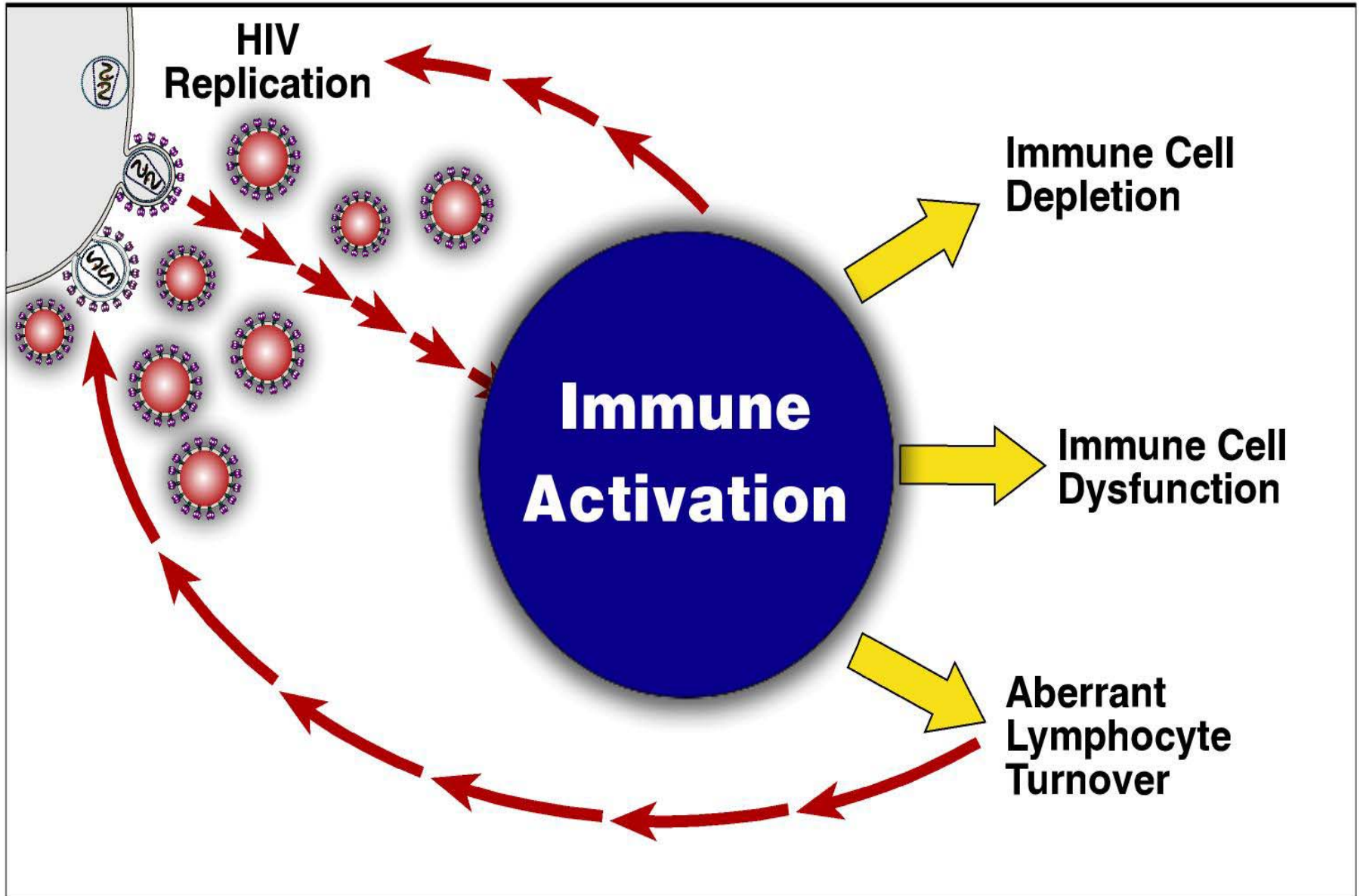
Pathogenic Mechanisms of HIV Disease



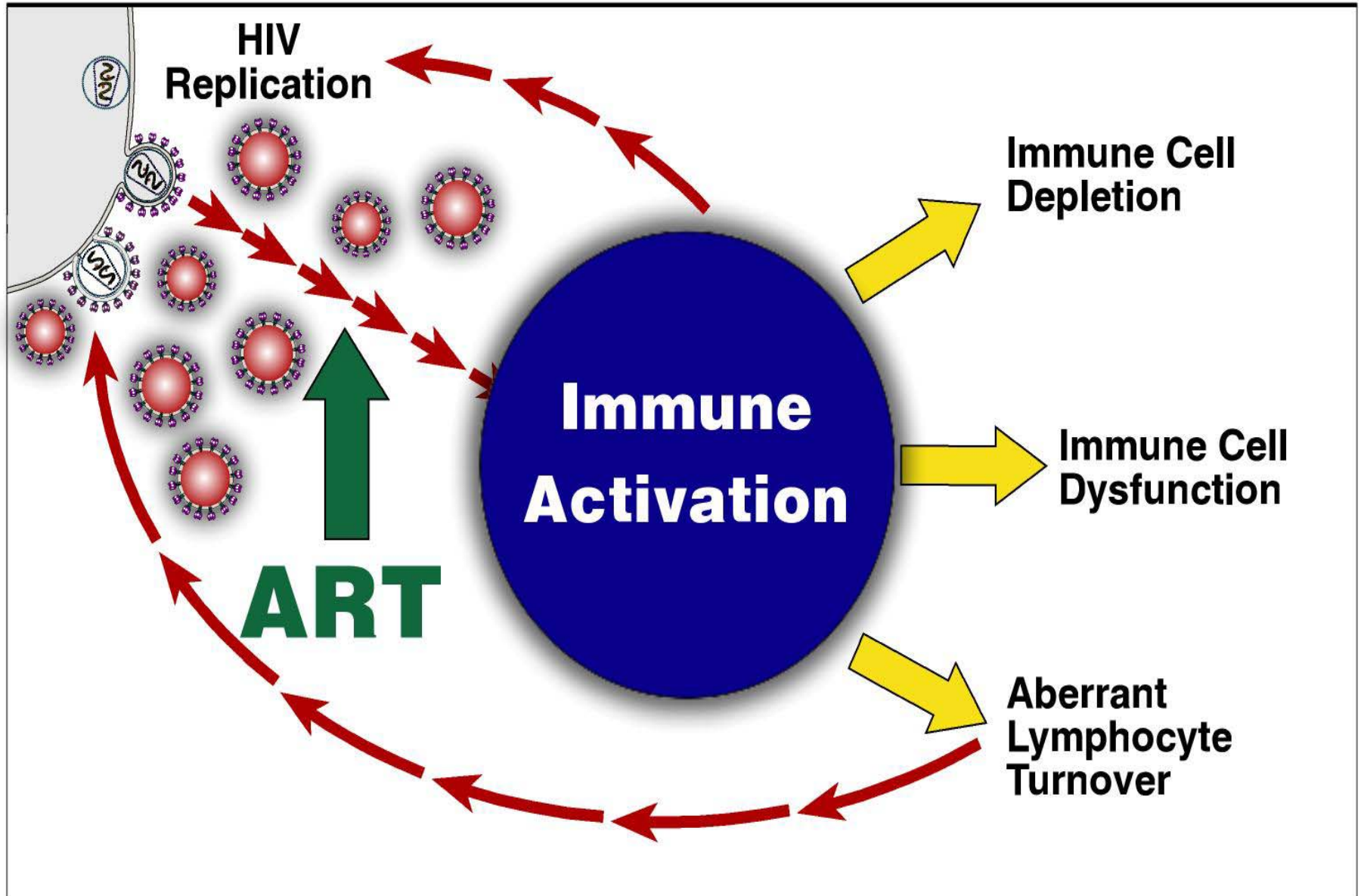
Pathogenic Mechanisms of HIV Disease



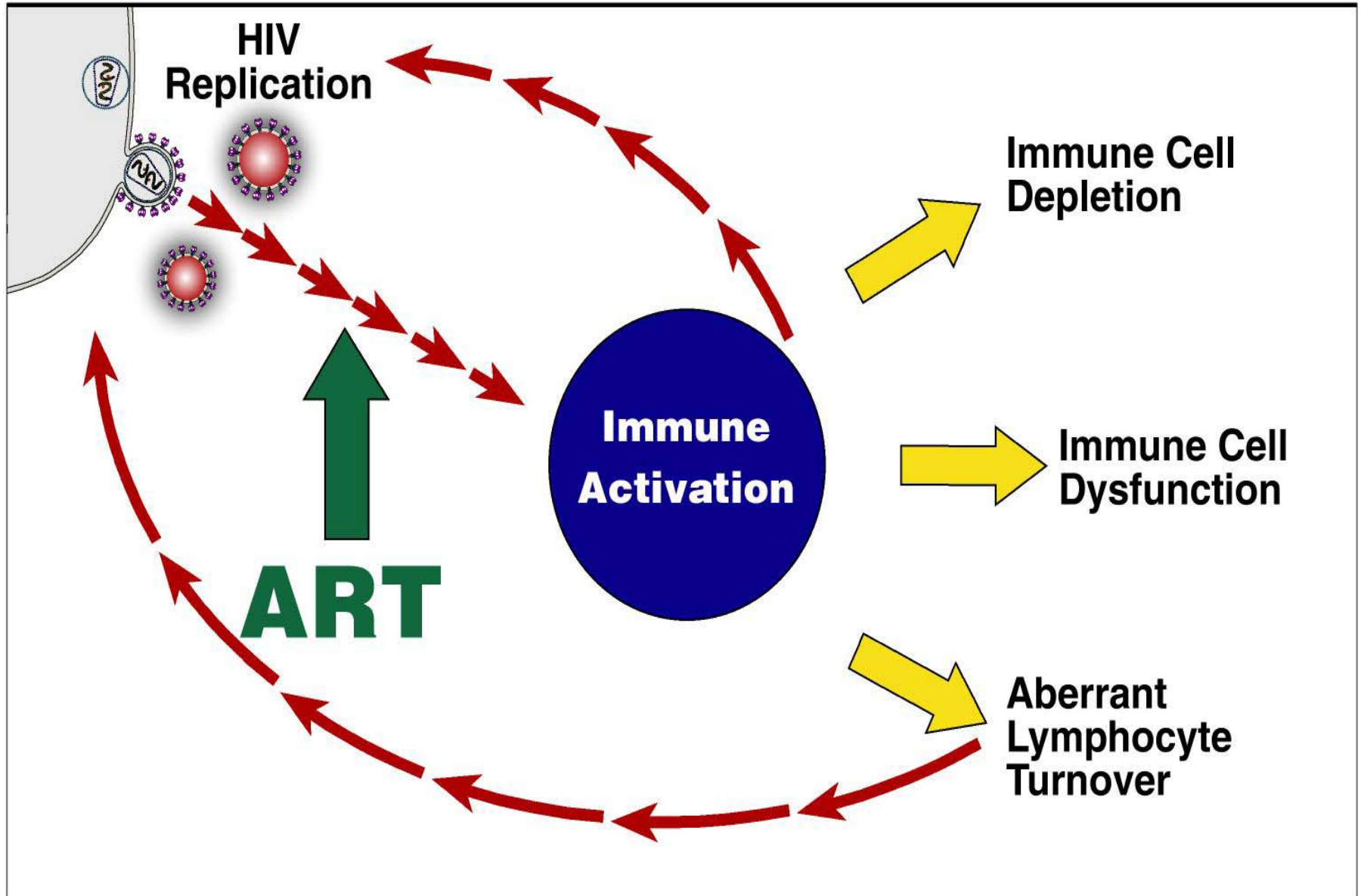
Pathogenic Mechanisms of HIV Disease



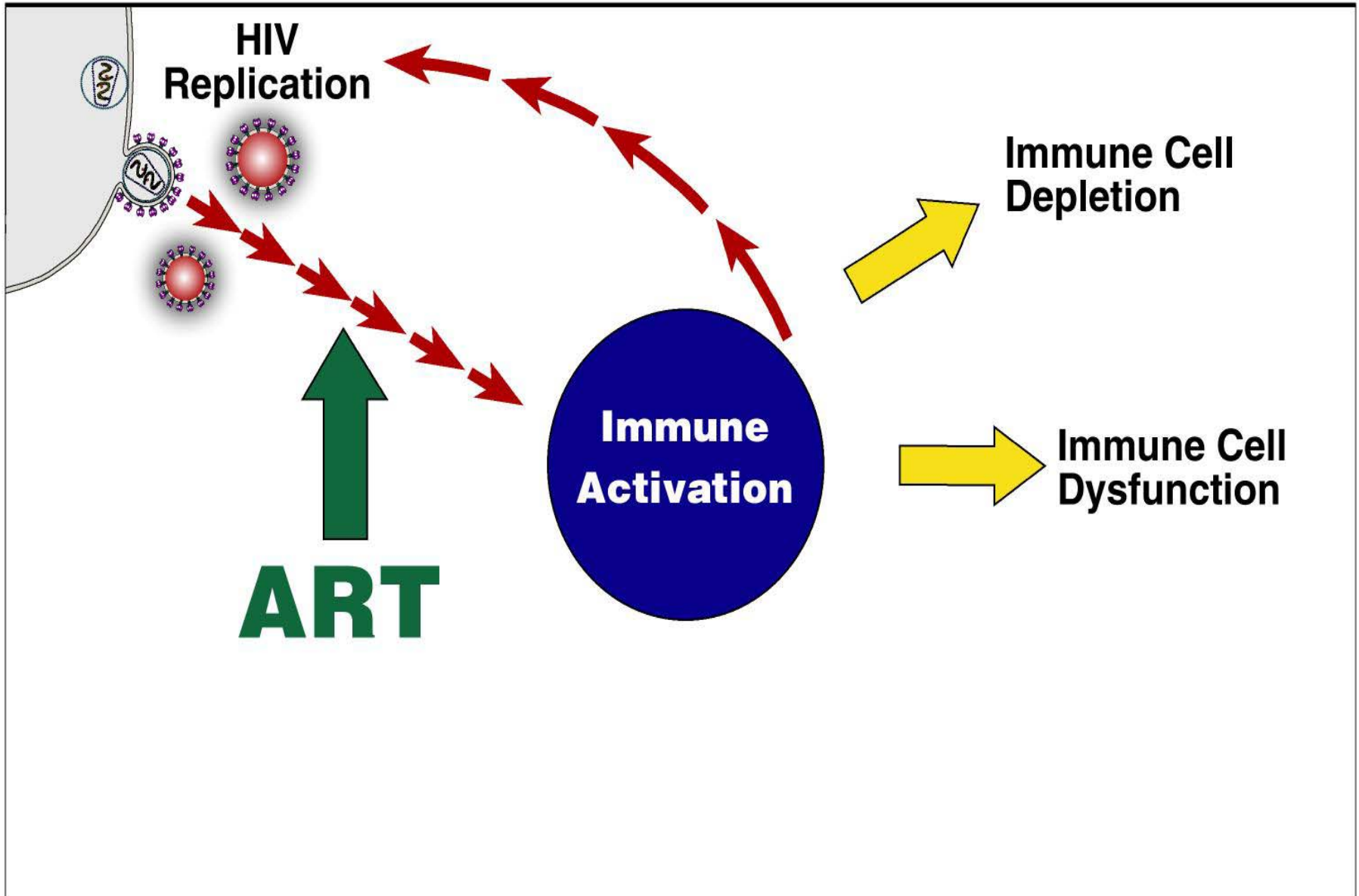
Pathogenic Mechanisms of HIV Disease



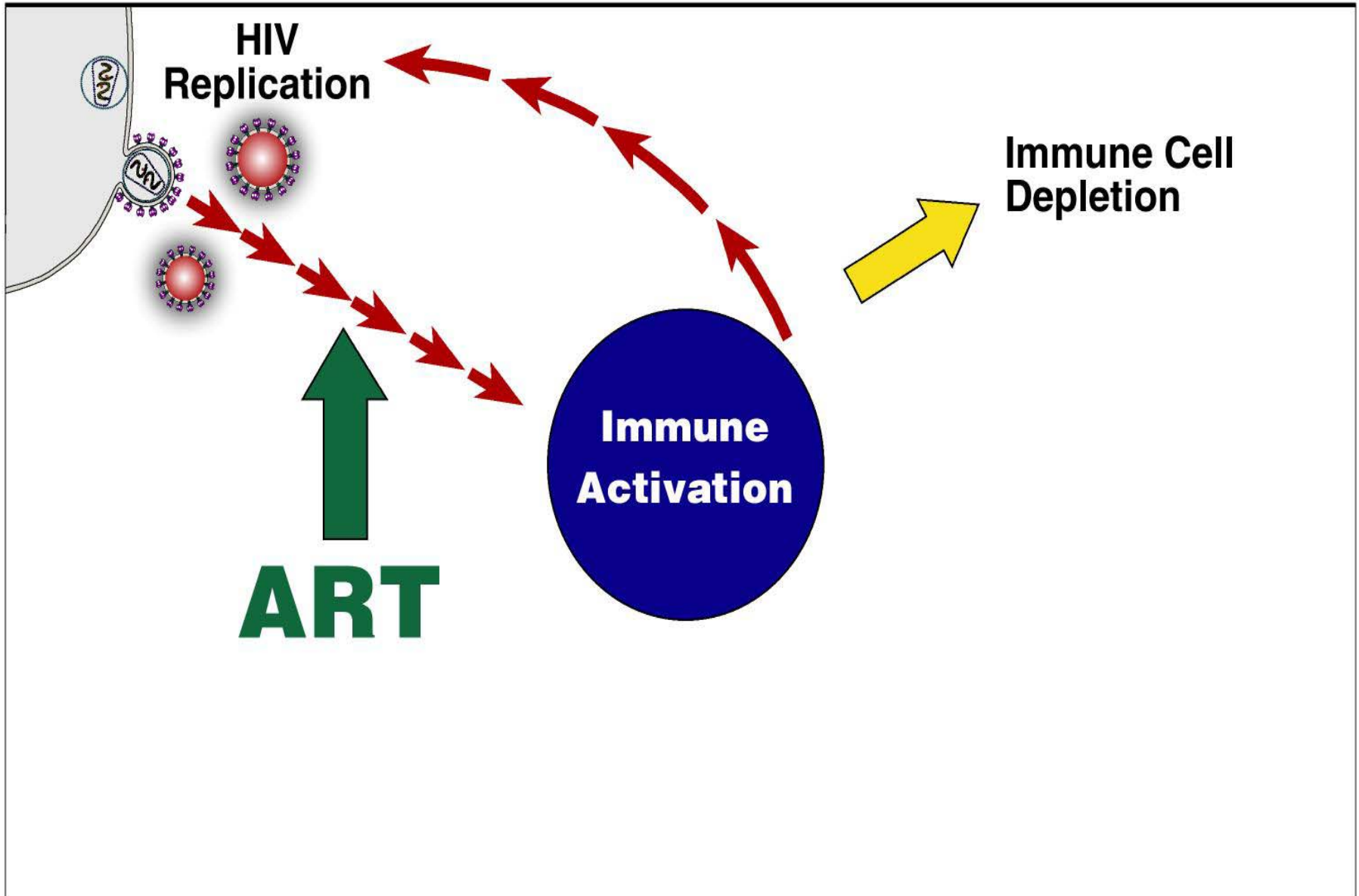
Pathogenic Mechanisms of HIV Disease



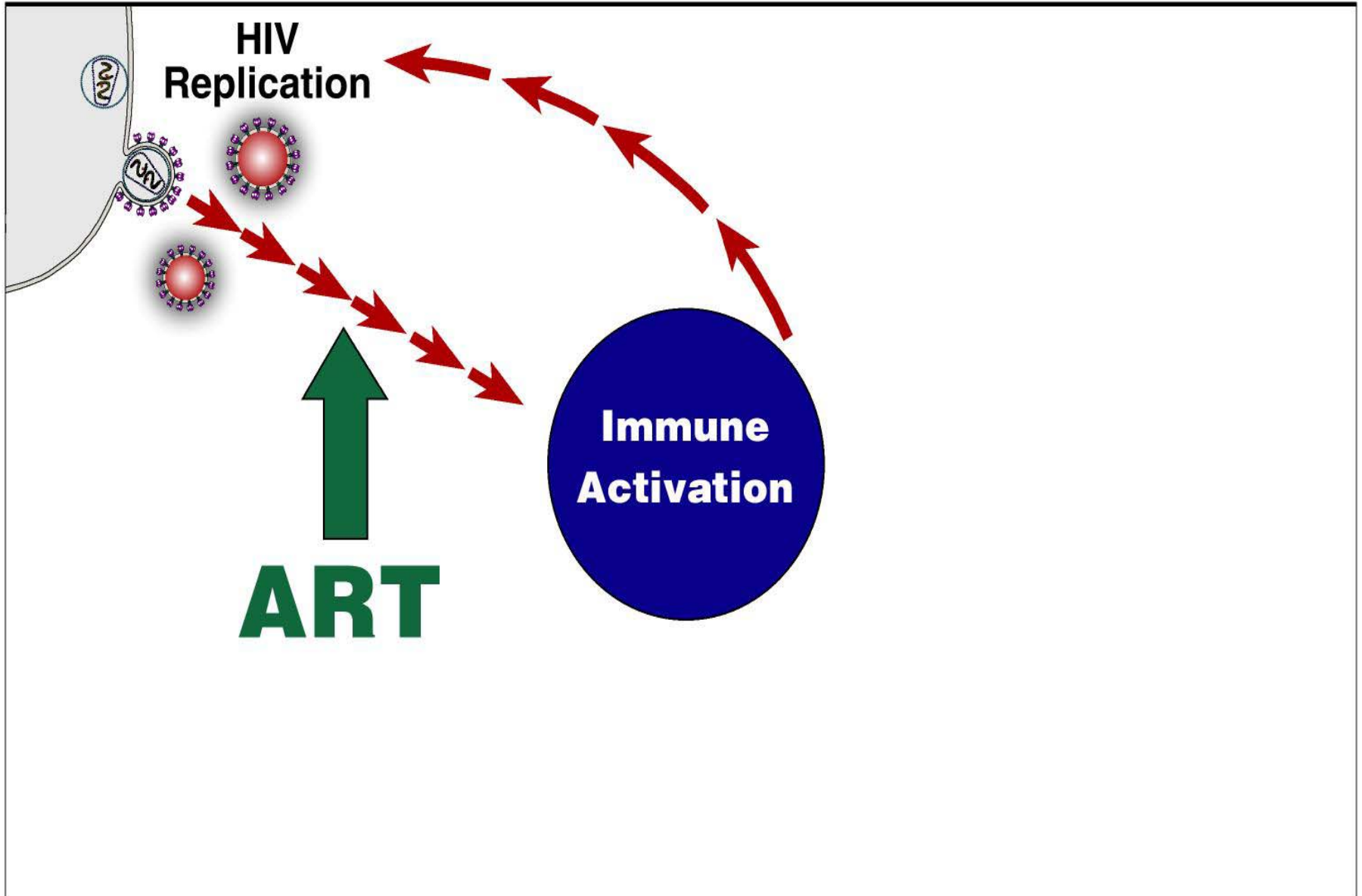
Pathogenic Mechanisms of HIV Disease



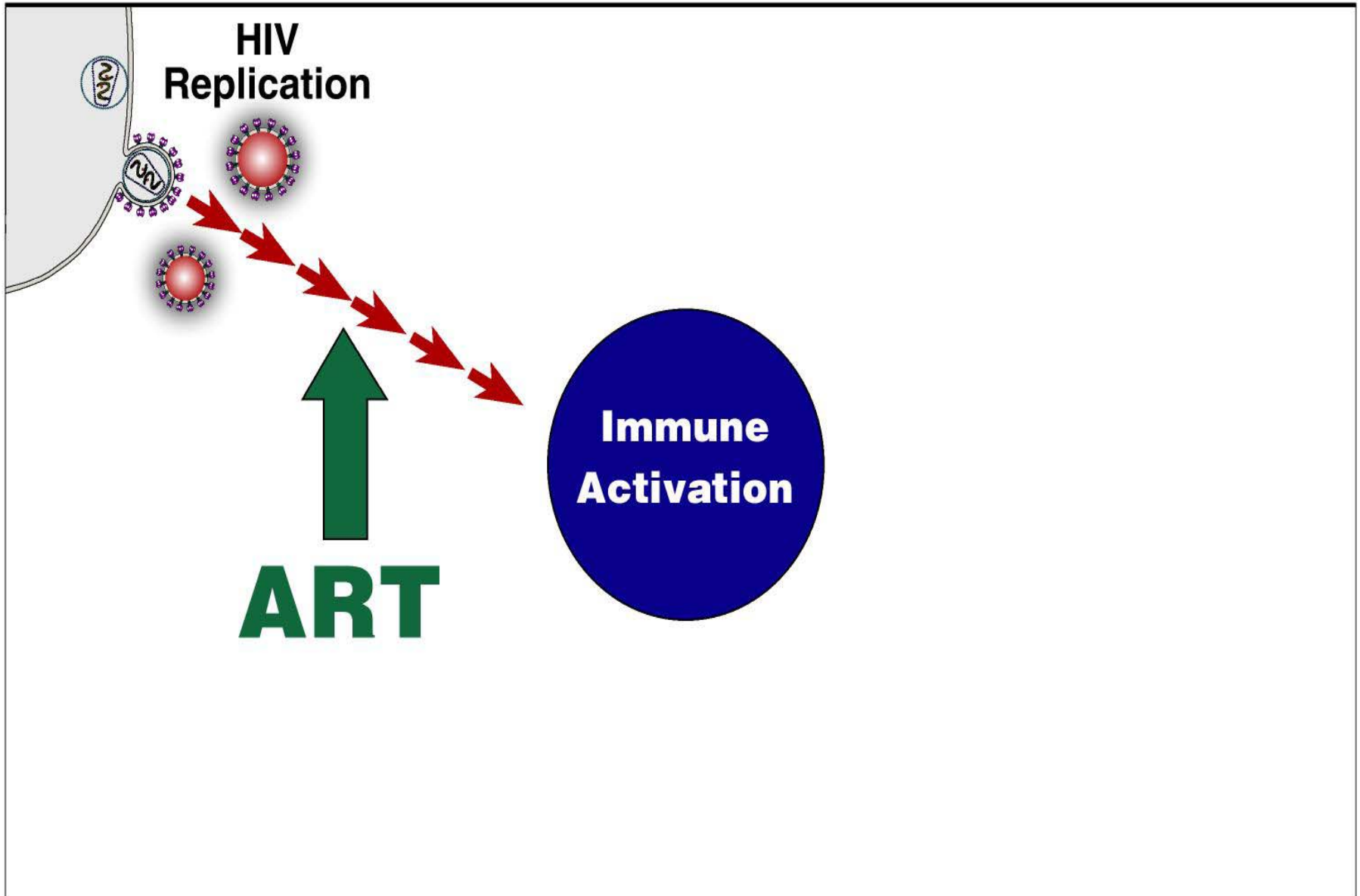
Pathogenic Mechanisms of HIV Disease



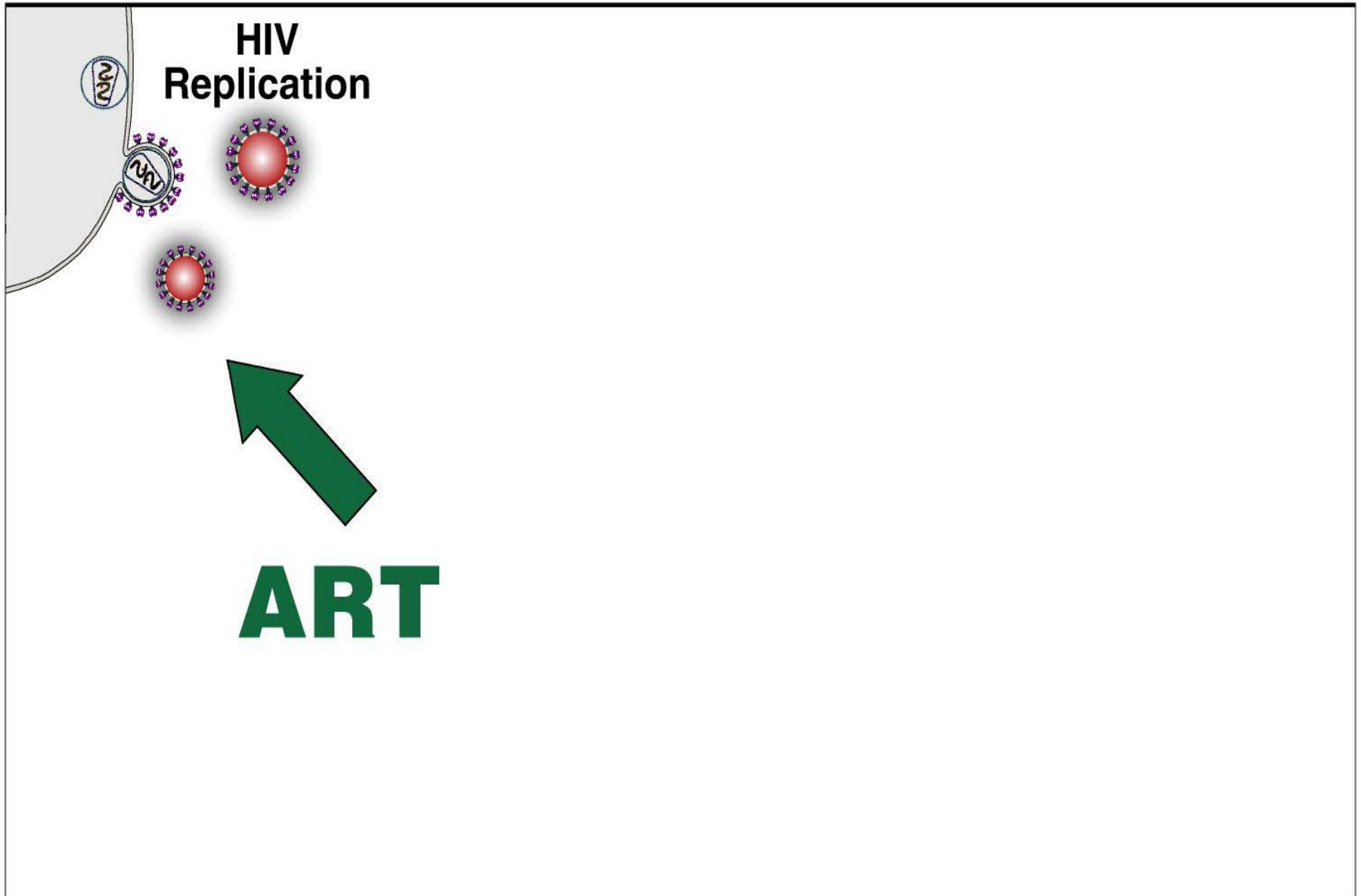
Pathogenic Mechanisms of HIV Disease



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Pathogenic Mechanisms of HIV Disease



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