Hematopoetic-stem-cell based therapy for HIV disease

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Evidence for the Cure of HIV Infection by CCR5Δ32/Δ32 Stem Cell Transplantation

Editorial
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Abbrevations

cAMP: Cyclic Adenosine Monophosphate; HSC: Hematopoetic Stem Cell; ICER: Inducible cAMP Early Repressor; CREM: Cyclic AMP Responsive Element Modulator; TFs: Transcription Factors; NFATc1: Nuclear Factor of Activated T Cell c1; nTreg: Naturally Occurring Regulatory T Cells; Tcons: Conventional CD4+ T Cells

Editorial

The unprecedented power of the hematopoetic stem cell (HSC) transplantation of the CCR5Δ32/Δ32 cells resistant to HIV has been proven to cure HIV infection in the case of leukemic patient ('Berlin patient' – Timothy Ray Brown) reported two years ago [1]. Since then another two cases proven to cure HIV infection after hematopoetic stem cell (HSC) transplantation of the CCR5Δ32/Δ32 cells were reported from Brigham and Women Hospital in Boston [2].

The Δ32 mutation at the CCR5 locus was found in Europe, with higher frequencies in the north [3]. Homozyous carriers of the Δ32 mutation (CCR5Δ32/Δ32) are resistant to HIV-1 infection because the mutation prevents functional expression of the CCR5 chemokine receptor used by HIV-1 to enter CD4+ T cells. The CCR5Δ32 mutation is a good example of an advantageous allele with a well-characterized geographic distribution. The Δ32 mutation currently plays an important role in HIV resistance because heterozygous carriers have reduced susceptibility to infection and delayed onset of AIDS, while homozygous carriers are resistant to HIV infection [4]. Bubonic plague was initially proposed as the selective agent, but the subsequent analysis suggested that a disease like smallpox is a more plausible candidate [5]. Lucotte and Mercier [6] suggested that the geographic distribution imply a Viking origin. In particular they proposed that the allele was present in Scandinavia before 1,000 to 1,200 years ago and then was carried by Vikings westward to Iceland, eastward to Russia, and southward to central and southern Europe.

The feasibility of this concept (Figure 1) is based on novel insights of the protection from GvHD by elevated levels of cAMP through binding of HIV-1 envelope protein gp120 to human regulatory T cells (Tregs) (Figure 2) [8,9]. Tregs represent a unique T-cell lineage endowed with the ability to effectively suppress immune responses. Therefore, approaches to modulate Treg function *in vivo* could provide ways to enhance or reduce immune responses and lead to novel therapies. It is known for a long time that Tregs need to be activated to exert their suppressive function on bystander conventional CD4+ T cells (Tcons). However, it has remained elusive how activation of Tregs may occur effectively, as their suppression is not restricted and their antigen specificity may be different from the cells they suppress. Earlier reports on anti-CD4-mediated tolerance and Treg activation took advantage of the HIV gp120 protein having been a high-affinity ligand for CD4 and reported that gp120 – mediated activation of naturally occurring Tregs (nTregs) through CD4 was sufficient to turn on the suppressive activity in nTregs (Figure 2) [10]. CD4-mediated activation depends on Lck and low levels of cyclic adenosine monophosphate (cAMP) production and can be blocked by Src family kinase inhibitors and adenylyl cyclase inhibitors. Functional analysis of the effects of gp120-mediated activation of Treg *in vivo* in a GvHD model demonstrated that the Treg activation by gp120 through adenylyl cyclase could abolish the rejection [8]. The data on gp120 are very important in the context Treg-mediated suppression *in vivo* as a starting point for potential new therapies to ameliorate GvHD in the course of HSC transplantation of the cells resistant to HIV-1. In terms of link

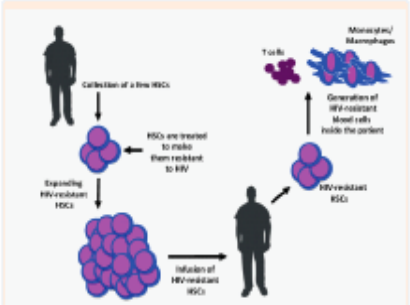
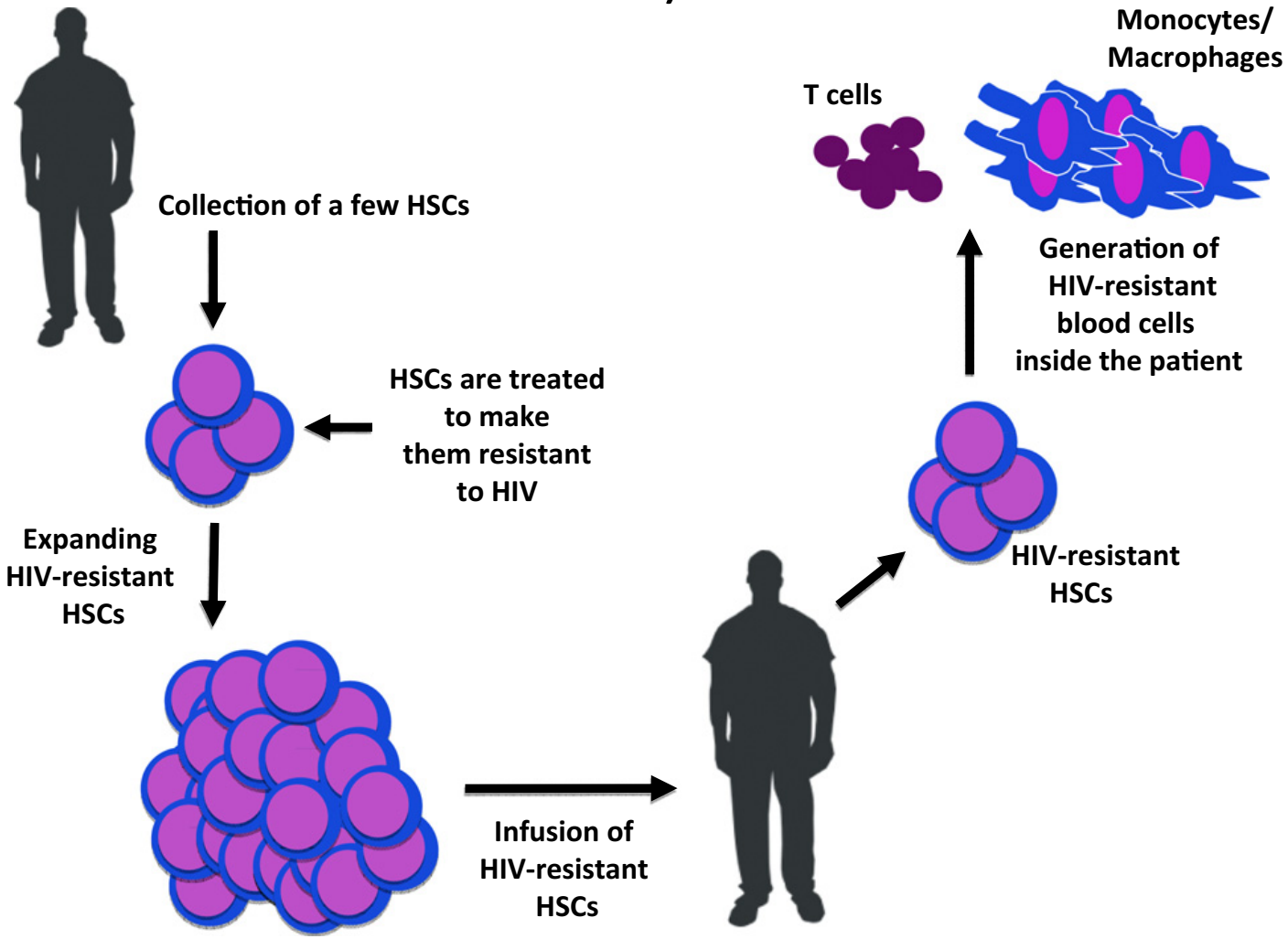
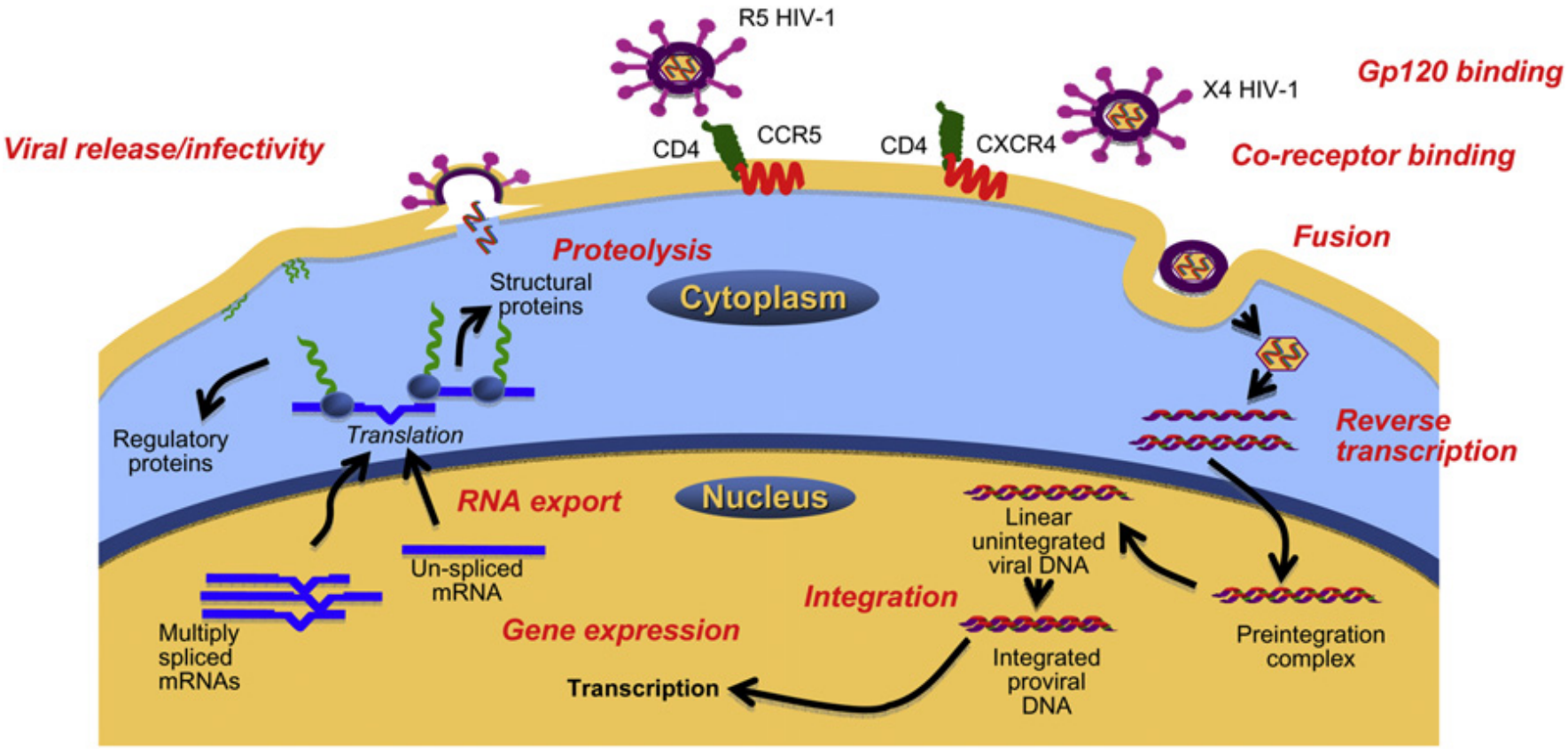


Figure 1: Hematopoetic-stem-cell based therapy for HIV disease (adopted from Klem et al. [7]. Long lived, self-renewing, multilineage hematopoetic stem cells (HSCs) selected such that their progeny resist HIV-1 infection (such as HSCs from donor harboring CCR5Δ32 mutation). The host could thereafter be repopulated with a hematopoetic system (including CD4+ T cells and myeloid targets for HIV) that is resistant to the replication and spread of HIV.

Intracellular immunization with gene-modified hematopoietic stem cells (Kiem et al. *Cell Stem Cell* 2012)



The replicative cycle of HIV (Kiem et al. Cell Stem Cell, 2012)



Identification of RANTES, MIP-1α, and MIP-1β as the Major HIV-Suppressive Factors Produced by $CD8^+$ T Cells

Fiorenza Cocchi,* Anthony L. DeVico, Alfredo Garzino-Demo, Suresh K. Arya, Robert C. Gallo,*† Paolo Lusso‡

Evidence suggests that $CD8^+$ T lymphocytes are involved in the control of human immunodeficiency virus (HIV) infection in vivo, either by cytolytic mechanisms or by the release of HIV–suppressive factors (HIV-SF). The chemokines RANTES, MIP-1α, and MIP-1β were identified as the major HIV-SF produced by $CD8^+$ T cells. Two active proteins purified from the culture supernatant of an immortalized $CD8^+$ T cell clone revealed sequence identity with human RANTES and MIP-1α. RANTES, MIP-1α, and MIP-1β were released by both immortalized and primary $CD8^+$ T cells. HIV-SF activity produced by these cells was completely blocked by a combination of neutralizing antibodies against RANTES, MIP-1α, and MIP-1β. Recombinant human RANTES, MIP-1α, and MIP-1β induced a dose-dependent inhibition of different strains of HIV-1, HIV-2, and simian immunodeficiency virus (SIV). These data may have relevance for the prevention and therapy of AIDS.

Science, December 1995

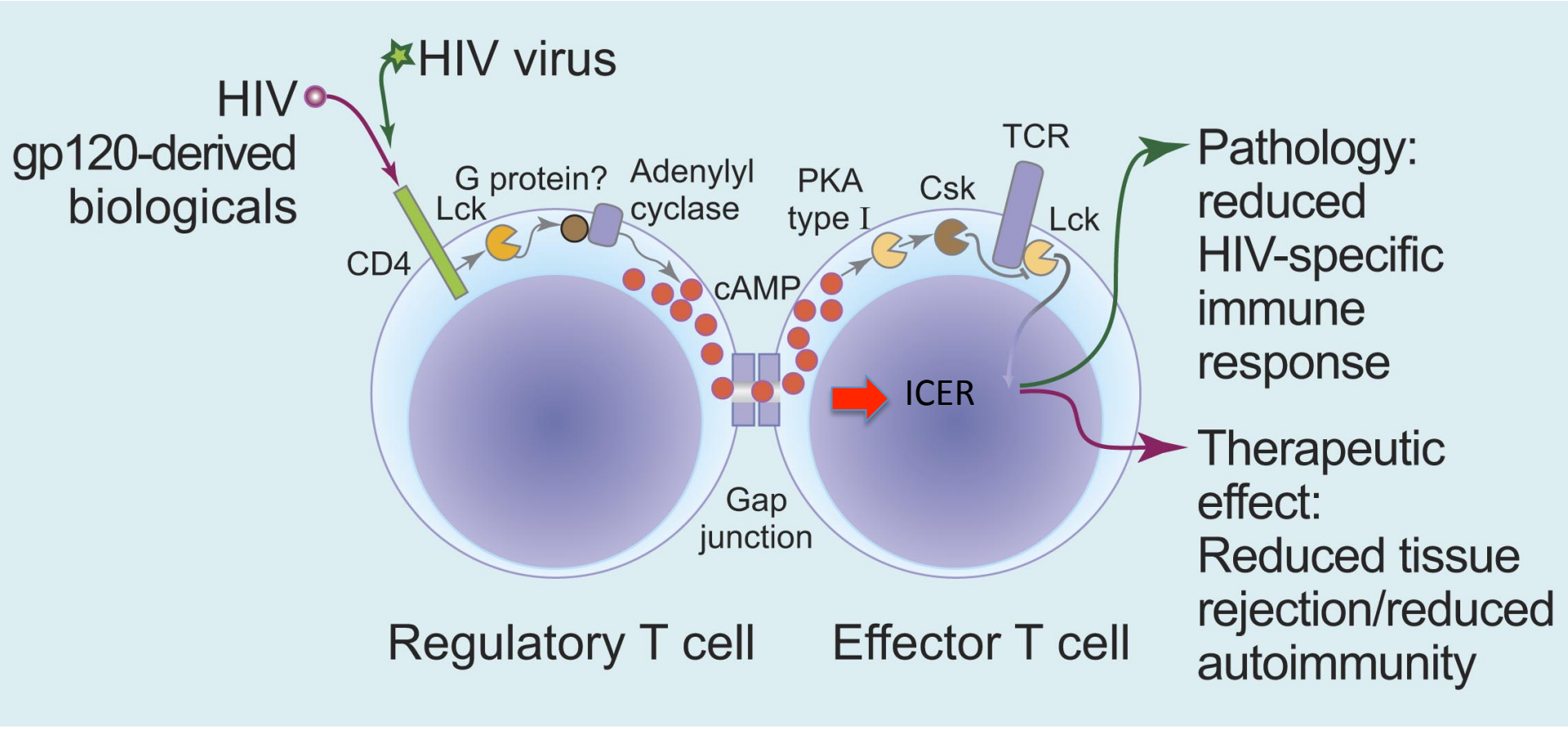
Genetic Acceleration of AIDS Progression by a Promoter Variant of *CCR5*

Maureen P. Martin, Michael Dean, Michael W. Smith, Cheryl Winkler, Bernard Gerrard, Nelson L. Michael, Benhur Lee, Robert W. Doms, Joseph Margolick,* Susan Buchbinder,† James J. Goedert,‡ Thomas R. O'Brien,‡ Margaret W. Hilgartner,§ David Vlahov,‖ Stephen J. O'Brien,¶ Mary Carrington

The *CCR5* gene encodes a cell surface chemokine receptor molecule that serves as the principal coreceptor, with CD4, for macrophage-tropic (R5) strains of human immunodeficiency virus–type 1 (HIV-1). Genetic association analysis of five cohorts of people with acquired immunodeficiency syndrome (AIDS) revealed that infected individuals homozygous for a multisite haplotype of the *CCR5* regulatory region containing the promoter allele, *CCR5P1*, progress to AIDS more rapidly than those with other *CCR5* promoter genotypes, particularly in the early years after infection. Composite genetic epidemiologic analyses of genotypes bearing *CCR5P1*, *CCR5-Δ32*, *CCR2-64I*, and *SDF1-3′A* affirmed distinct regulatory influences for each gene on AIDS progression. An estimated 10 to 17 percent of patients who develop AIDS within 3.5 years of HIV-1 infection do so because they are homozygous for *CCR5P1/P1*, and 7 to 13 percent of all people carry this susceptible genotype. The cumulative and interactive influence of these AIDS restriction genes illustrates the multigenic nature of host factors limiting AIDS disease progression.

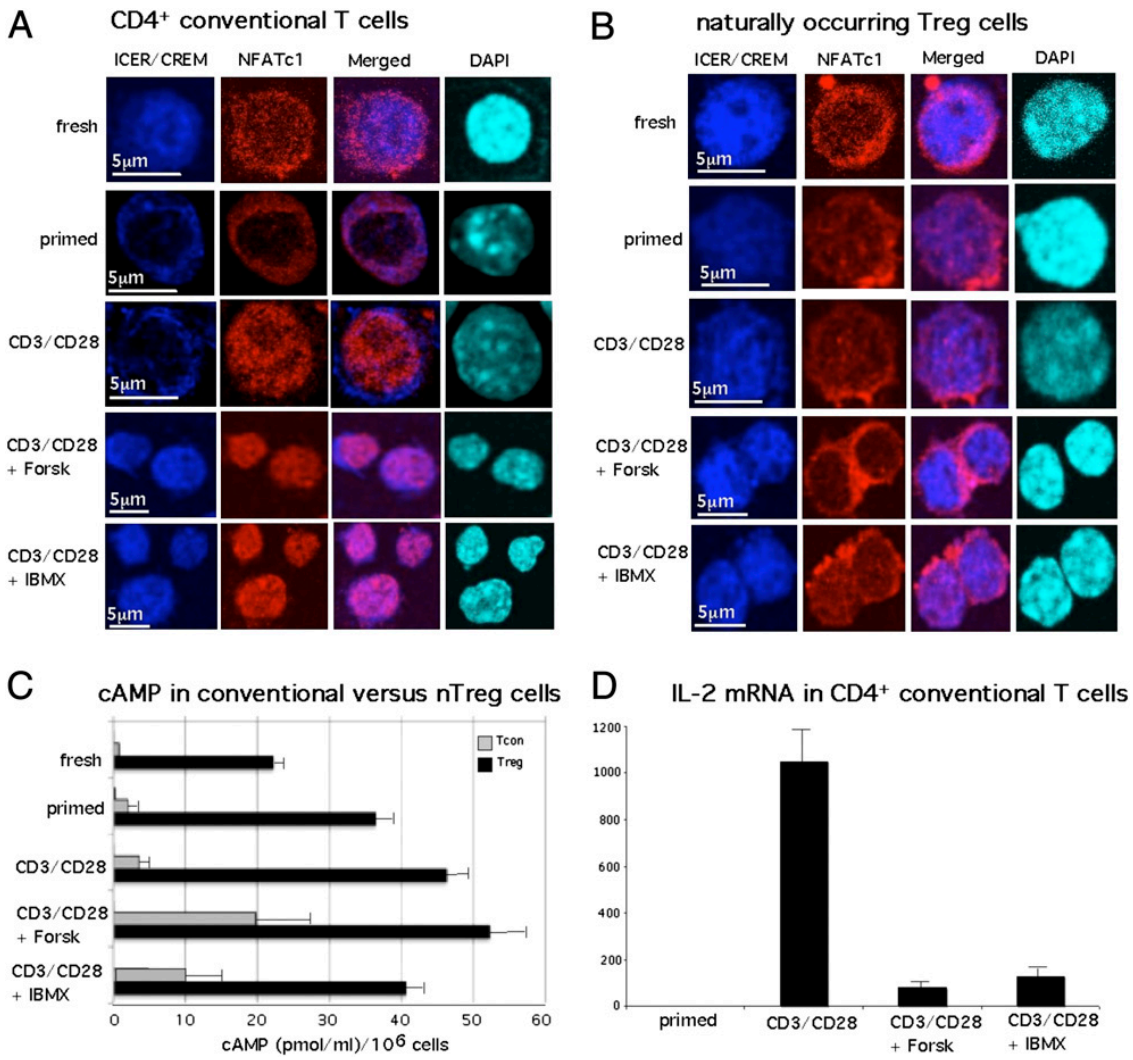
Science, December 1998

A schematic representation of Treg immunosuppression by cAMP following gp120 ligation to CD4

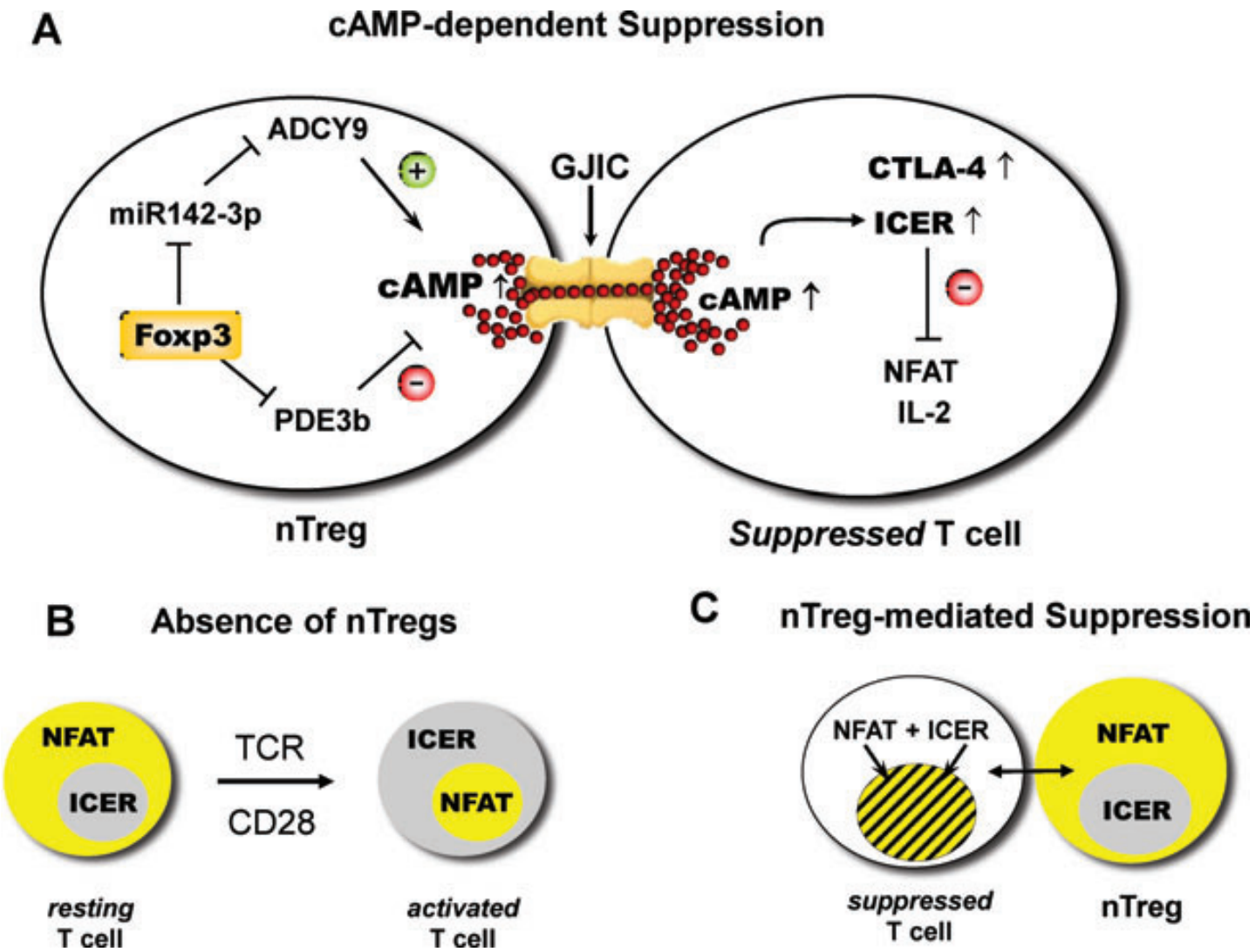


Adopted from Tasken, K., 2009, Blood, 114: 1136-7

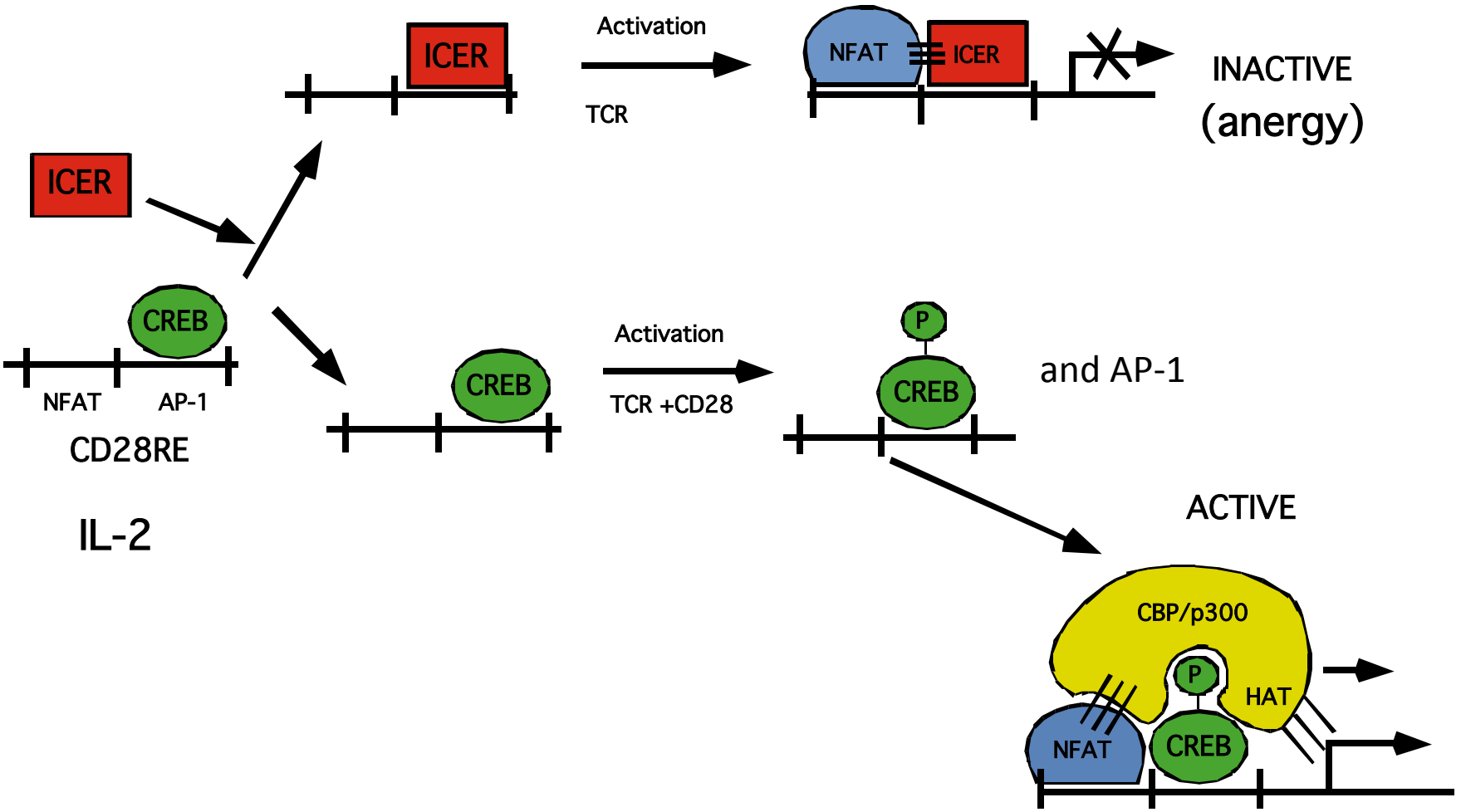
CD3/CD28 mAb stimulation directs ICER/CREM to the cytoplasm in conventional CD4+ T cells but not in Tregs



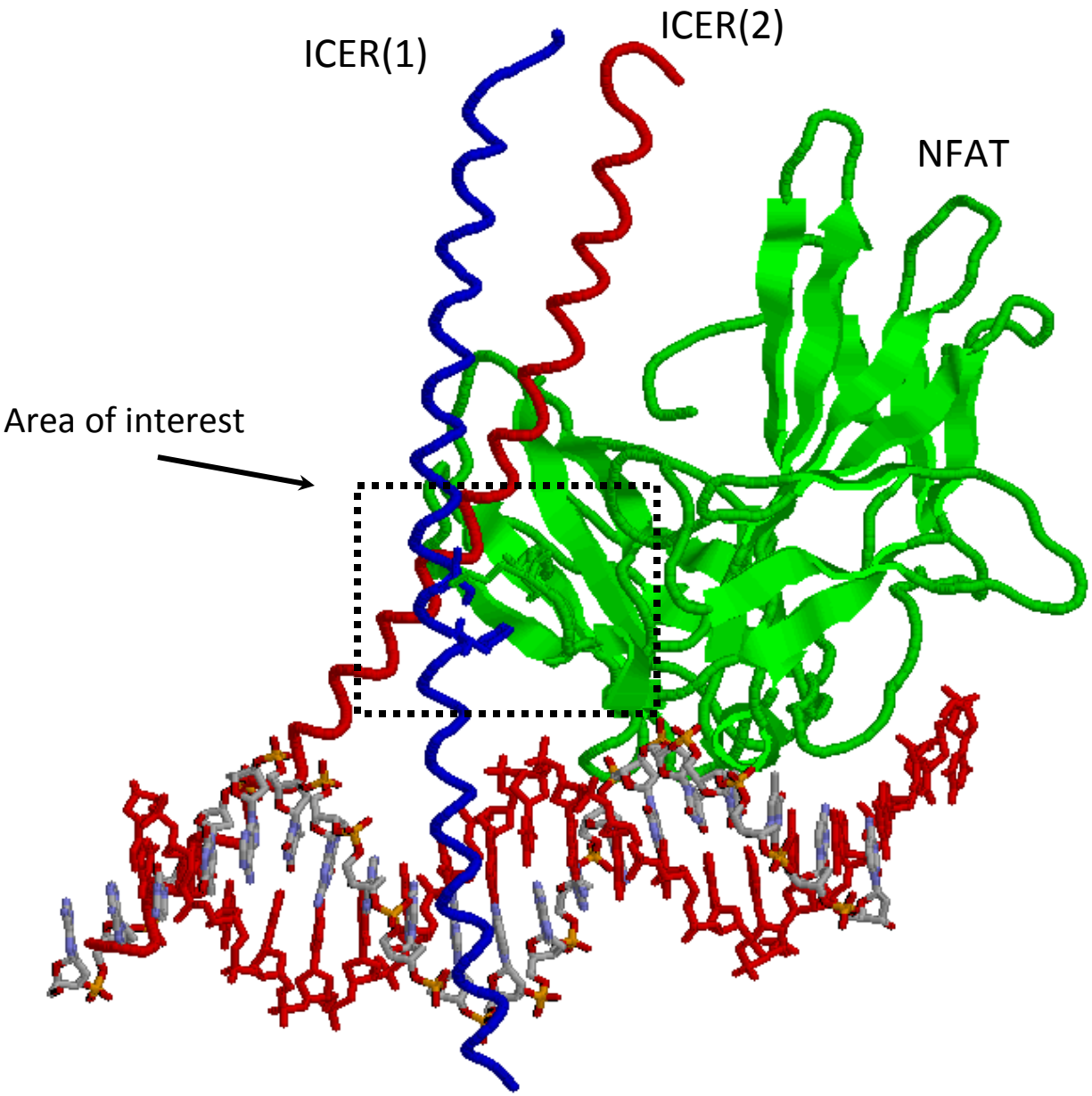
Treg cells direct ICER into the nucleus of activated conventional CD4$^+$ T cells via cAMP



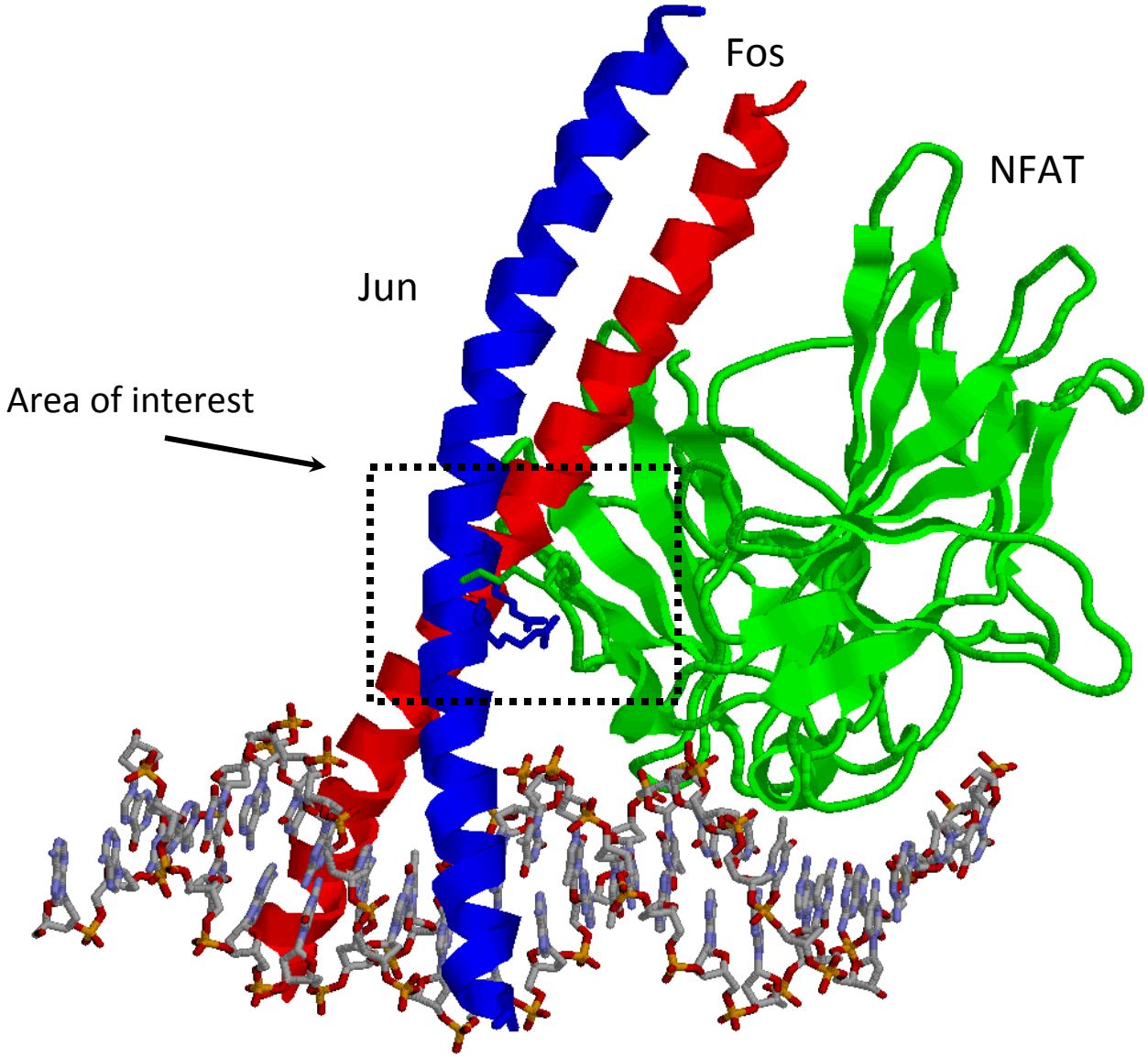
Model of CD28-responsive element (CD28RE) promoter regulation in the context of IL-2 promoter in the presence or absence of ICER



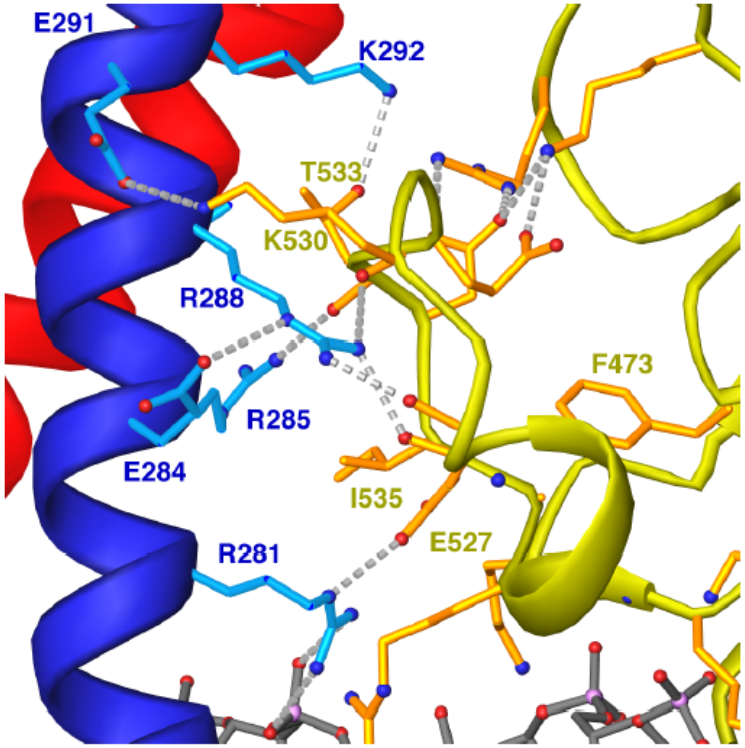
Modeled structure of the NFAT/ICER/DNA complex



Overall structure of the NFAT/Fos-Jun/DNA complex



Detailed interactions between NFAT (yellow)), Fos (red) and Jun (blue). The residues in Jun (labeled in blue letters) interacting with NFAT have functionally equivalent residues in c-Maf and ICER (blocked in yellow in the sequence alignment below).

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                                  281  285  288  292
C-Jun      KAERKRMRNRIAASKCRKRKLERIARLEEKVKTLKAQNSELASTANMLRE
C-Maf      KQKRRTLKNRGYAQSCRFKRVQQRHVLESEKNQLLQQVDHLKQEISRLVR
ICER       KRELRLMKNRFAAKECRRRKKEYVKCLESRVAVLEVQNKKLIEELETLKD
           * : : ::**  *..** :: :    **..   *  * ..* .  . *
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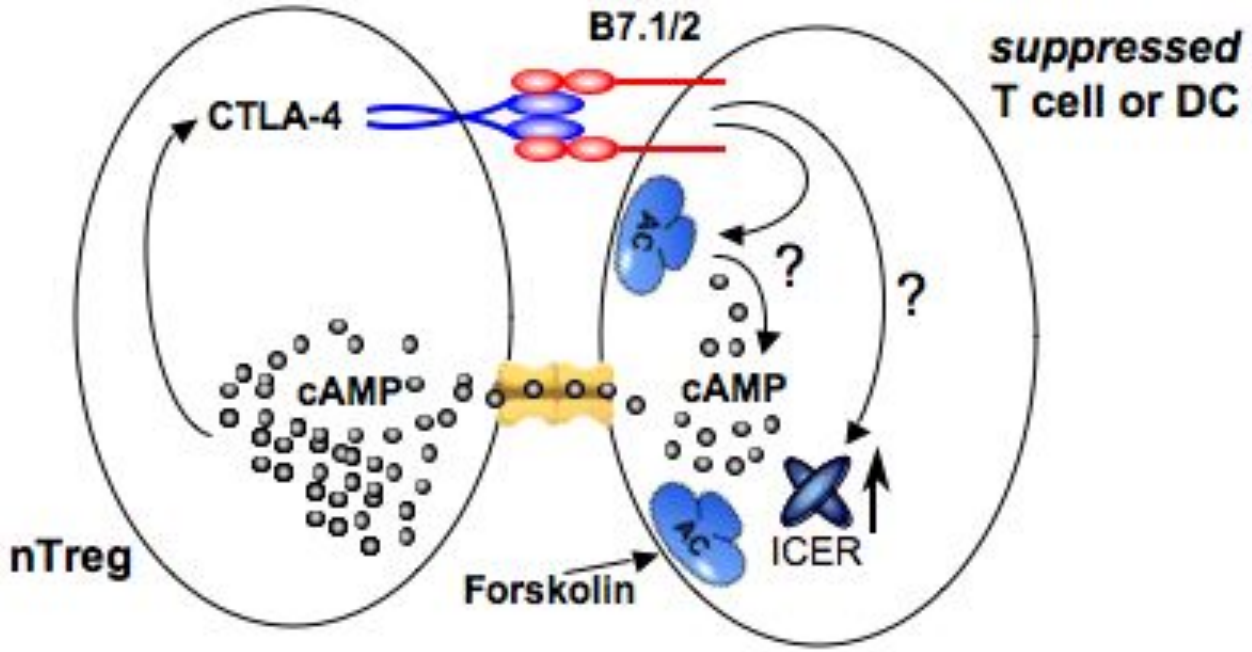
cAMP underpins suppression by Tregs



Prostaglandin-modulated umbilical cord blood hematopoietic stem cell transplantation

Corey Cutler,[1,2] Pratik Multani,[3] David Robbins,[3] Haesook T. Kim,[1] Thuy Le,[3] Jonathan Hoggatt,[2,4] Louis M. Pelus,[5] Caroline Desponts,[3] Yi-Bin Chen,[4] Betsy Rezner,[3] Philippe Armand,[1] John Koreth,[1] Brett Glotzbecker,[1] Vincent T. Ho,[1] Edwin Alyea,[1] Marlisa Isom,[1] Grace Kao,[1] Myriam Armant,[6] Leslie Silberstein,[2,6] Peirong Hu,[5] Robert J. Soiffer,[1] David T. Scadden,[2,4] Jerome Ritz,[1] Wolfram Goessling,[1,2,7] Trista E. North,[2,8,1] John Mendlein,[3] Karen Ballen,[4] Leonard I. Zon,[1,2,8,9] Joseph H. Antin,[1] and Daniel D. Shoemaker[3]

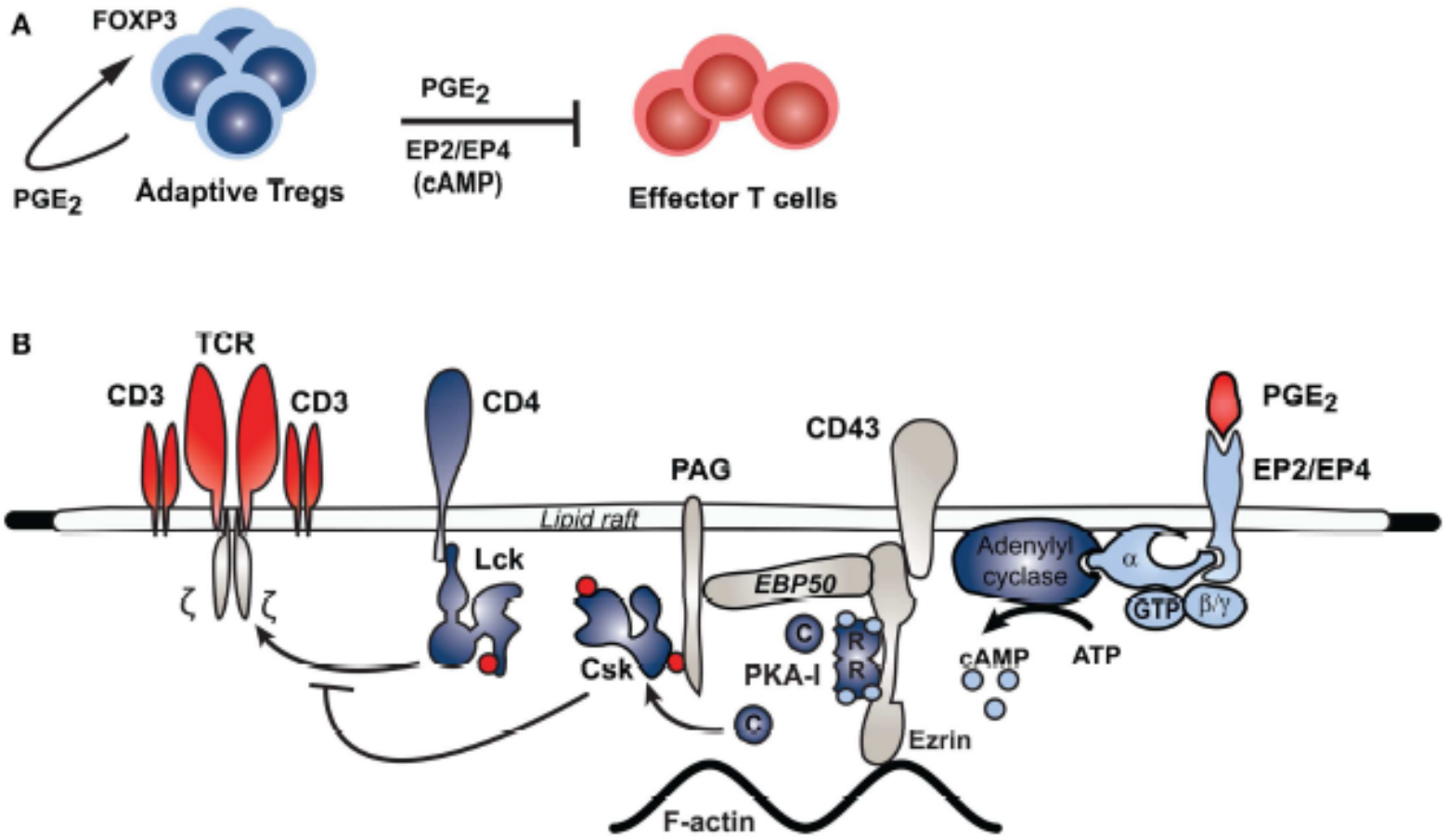
[1]Dana-Farber Cancer Institute, Boston, MA; [2]Harvard Stem Cell Institute, Cambridge, MA; [3]Fate Therapeutics, San Diego, CA; [4]Massachusetts General Hospital, Boston, MA; [5]Indiana University, Indianapolis, IN; [6]Children's Hospital, Boston, MA; [7]Brigham and Women's Hospital, Boston, MA; [8]Beth Israel Deaconess Medical Center, Boston, MA; and [9]Howard Hughes Medical Institute, Chevy Chase, MD

Key Points

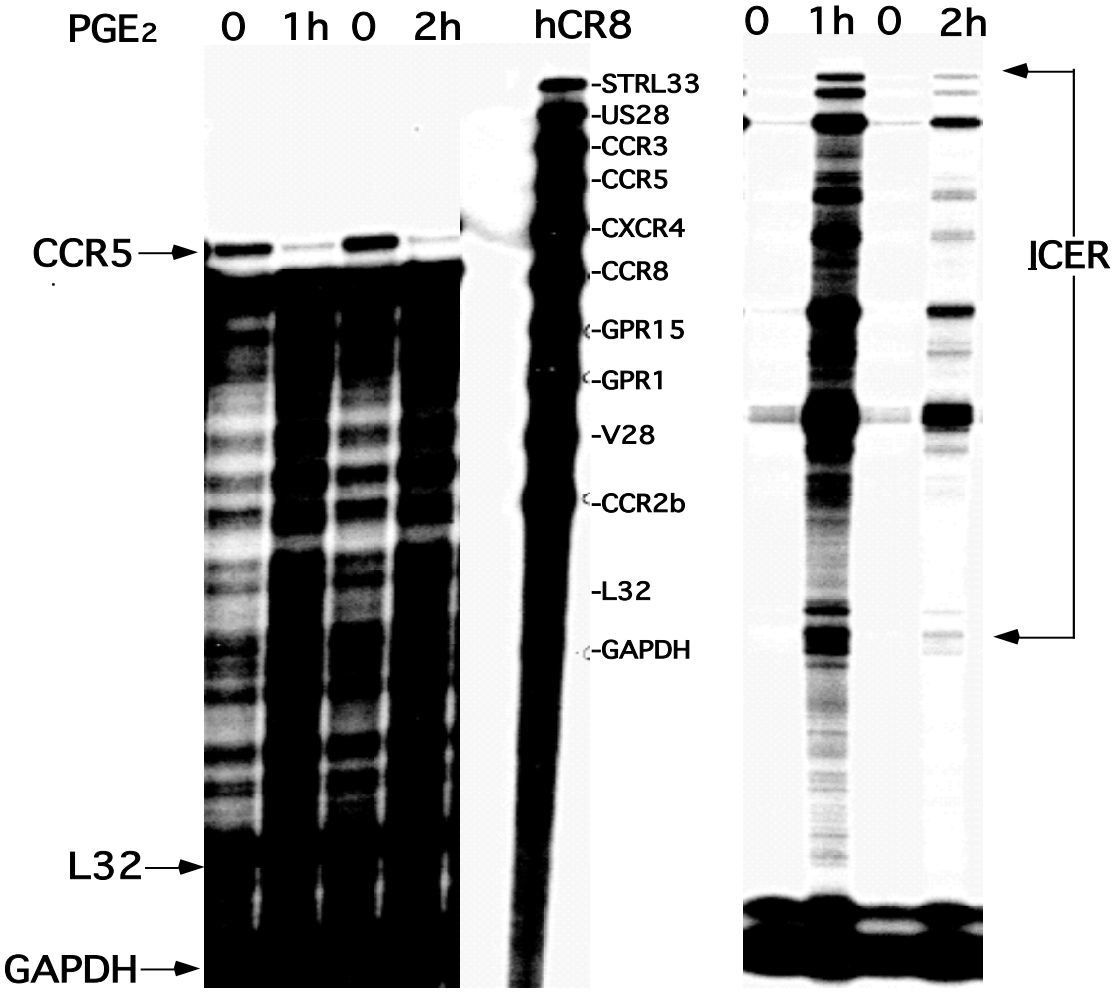
- Molecular profiling was used to optimize an ex vivo modulation protocol with $dmPGE_2$ for UCB transplantation.
- Pulse treatment of UCB with $dmPGE_2$ is safe and may lead to accelerated UCB engraftment and preferential cord chimerism.

Umbilical cord blood (UCB) is a valuable source of hematopoietic stem cells (HSCs) for use in allogeneic transplantation. Key advantages of UCB are rapid availability and less stringent requirements for HLA matching. However, UCB contains an inherently limited HSC count, which is associated with delayed time to engraftment, high graft failure rates, and early mortality. 16,16-Dimethyl prostaglandin E_2 ($dmPGE_2$) was previously identified to be a critical regulator of HSC homeostasis, and we hypothesized that brief ex vivo modulation with $dmPGE_2$ could improve patient outcomes by increasing the "effective dose" of HSCs. Molecular profiling approaches were used to determine the optimal ex vivo modulation conditions (temperature, time, concentration, and media) for use in the clinical setting. A phase 1 trial was performed to evaluate the safety and therapeutic potential of ex vivo modulation of a single UCB unit using $dmPGE_2$ before reduced-intensity, double UCB transplantation. Results from this study demonstrated clear safety with durable, multilineage engraftment of $dmPGE_2$-treated UCB units. We observed encouraging trends in efficacy, with accelerated neutrophil recovery (17.5 vs 21 days, $P = .045$), coupled with preferential, long-term engraftment of the $dmPGE_2$-treated UCB unit in 10 of 12 treated participants. This study was registered at www.clinicaltrials.gov as #NCT00890500. (*Blood*. 2013;122(17):3074-3081)

Inhibitory pathway of PGE_2 in effector T cells



PGE_2-mediated transcriptional attenuation of CCR5 correlates with ICER induction



Suppression of MIP-1β transcription in human T cells is regulated by inducible cAMP early repressor (ICER)

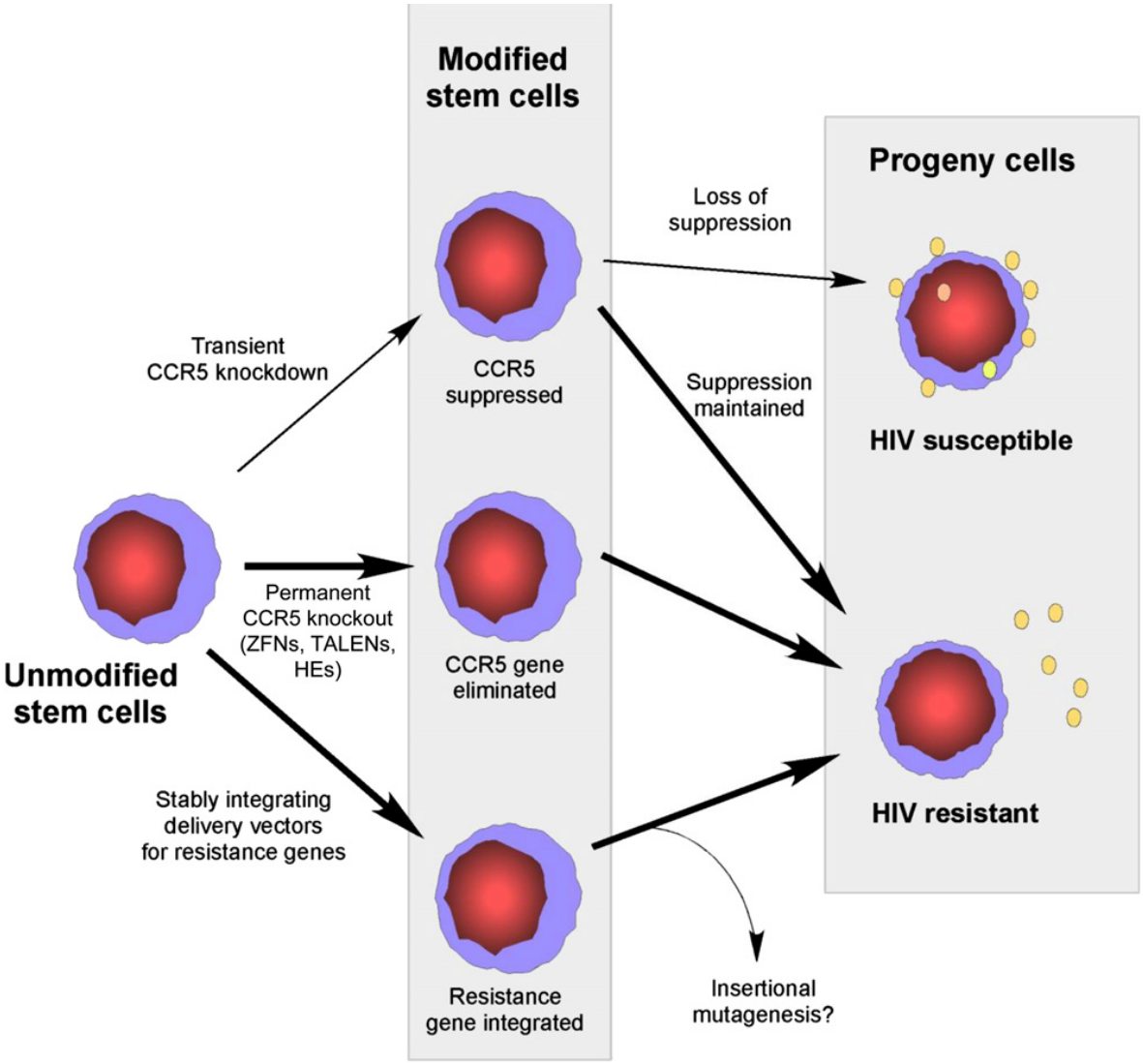
Oxana Barabitskaja,* James S. Foulke Jr.,* Shibani Pati,* Josef Bodor,† and Marvin S. Reitz Jr.*,‡,1

*Institute of Human Virology, University of Maryland Biotechnology Institute, and ‡Department of Microbiology and Immunology, University of Maryland, Baltimore; †Department of Pathology, Columbia University, New York, New York

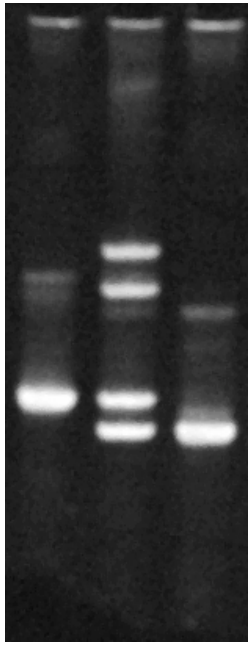
Abstract: Local production of macrophage inflammatory protein-1β (MIP-1β), a β-chemokine that blocks human immunodeficiency virus type 1 (HIV-1) entry into CD4+ CC chemokine receptor 5+ target cells, may be a significant factor in resistance to HIV-1 infection and control of local viral spread. The mechanisms governing MIP-1β expression in T cells, however, are not well understood. Our results suggest that MIP-1β RNA expression in T cells is dynamically regulated by transcriptional factors of the cyclic adenosine monophosphate (cAMP) responsive element (CRE)-binding (CREB)/modulator family. Transient transfection of primary human T cells with 5′ deletion and site-specific mutants of the human MIP-1β promoter identified an activated protein-1 (AP-1)/CRE-like motif at position −74 to −65 base pairs, relative to the TATA box as a vital cis-acting element and a binding site for inducible cAMP early repressor (ICER). Ectopic expression of ICER or induction of endogenous ICER with the cAMP agonists forskolin and prostaglandin E_2 resulted in the formation of ICER-containing complexes, including an ICER:CREB heterodimer to the AP-1/CRE-like site and inhibition of MIP-1β promoter activity. Our data characterize an important binding site for the dominant-negative regulator ICER in the MIP-1β promoter and suggest that dynamic changes in the relative levels of ICER and CREB play a crucial role in cAMP-mediated attenuation of MIP-1β transcription in human T cells. *J. Leukoc. Biol.* 79: 378–387; 2006.

J. Leukoc. Biol. 2006

Approaches to modifying hematopoietic stem cells for HIV resistance (Kiem et al. Cell Stem Cell, 2012)



CCR5 genotyping



CCR5 genotyping. CCR5 expressing cells (wild type; the first lane) or cells with Δ32 mutation (heterozygous; the second lane) and cells homozygous for Δ32 mutation (CCR5Δ32/Δ32; the third lane) were detected using PCR. Genomic DNA was extracted from cells isolated from peripheral blood mononuclear cells and subjected to CCR5-specific PCR spanning the Δ32 region. To study the CCR5 gene variant in HIV target cells, genomic DNA was extracted and subjected to PCR amplification with primers for the CCR5 gene spanning the Δ32 region from nucleotide 826 to 1138 on the chromosome 3p21.31 (accession no: NM_000579). The expected fragments were 312 bp for the CCR5 wild-type (wt) and 280 pb for the Δ32 variant. These fragments (denoted by arrows) have been excised from the native acrylamide gel and individually sequenced to confirm exact location of Δ32 deletion.

Homozygous Defect in HIV Coreceptor CCR5 (Landau et al. 1996 Cell 86:367)

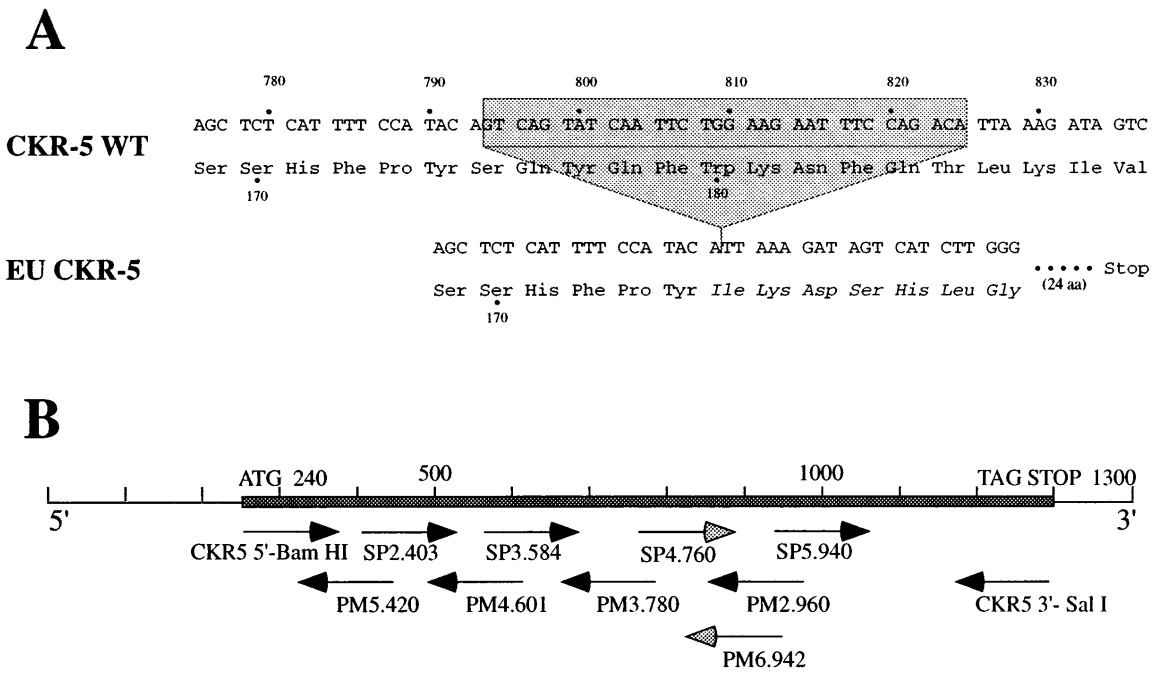


Figure 3. EU *CKR-5* Contains a 32 bp Internal Deletion

(A) The nucleotide sequences of wild-type *CKR-5* derived from normal donor (above) and EU cDNAs (below) are shown. The nucleotide sequence from normal donor *CKR-5* cDNA was determined for the complete 1055 bp coding sequence and found to be identical to that reported by Samson et al. (1996). The nucleotide sequence of 2 EU *CKR-5* cDNAs were identical to wild-type over the complete coding region with the exception of a 32 bp deletion. Nucleotide sequence of the 200 bp region of *CKR-5* encompassing the deletion was also determined for cDNAs derived from clones EU2.11, EU2.15, EU2.16, EU2.17, and EU3.1. Each contained the identical deletion with no additional nucleotide changes. Only the region flanking the deletion is shown. The deleted region (nt 794 to 825) is shaded. Amino acids encoded out of frame as a result of the deletion beginning at codon 185 are shown in italics. Nucleotide numbering (above) is from the first nucleotide of the reported *CKR-5* sequence (Samson et al., 1996). Amino acid residue numbers are shown below.

(B) Oligonucleotide primers used for nucleotide sequencing of *CKR-5* cDNA and detecting the deleted allele by PCR. The coding region is indicated by dark shading. Primers have been designated by nucleotide position at which they hybridize. Oligonucleotides used in PCR for detecting the deletion are shown as lightly shaded arrows. Oligonucleotides for cloning the full-length cDNA are shown at either end.

The Geographic Spread of the CCR5 Δ32 HIV-Resistance Allele (Novembre et al.2005, Plos Biol 3(11): e339)

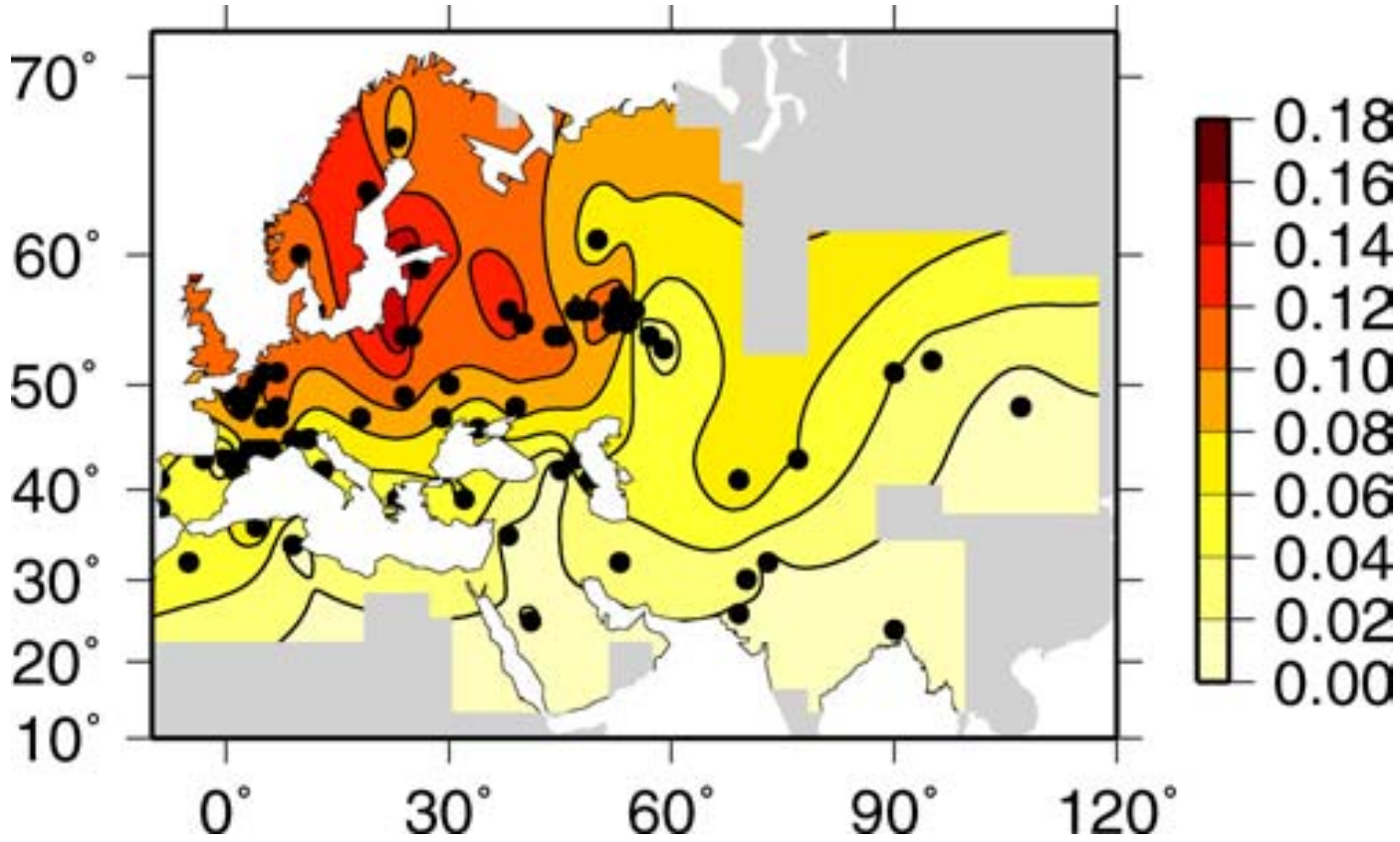
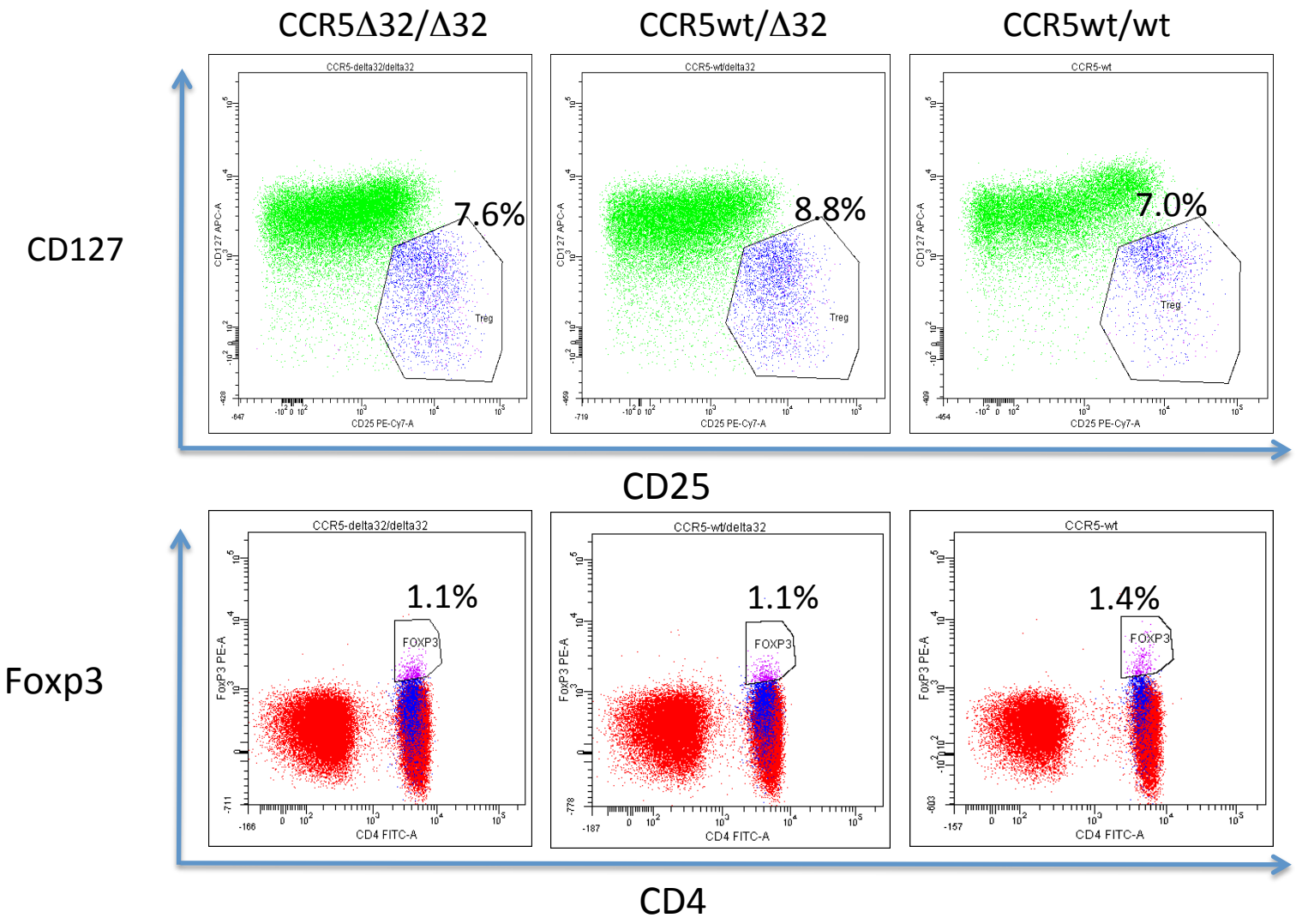


Figure 1. Shaded Contour Map of Δ32 Allele Frequency Data

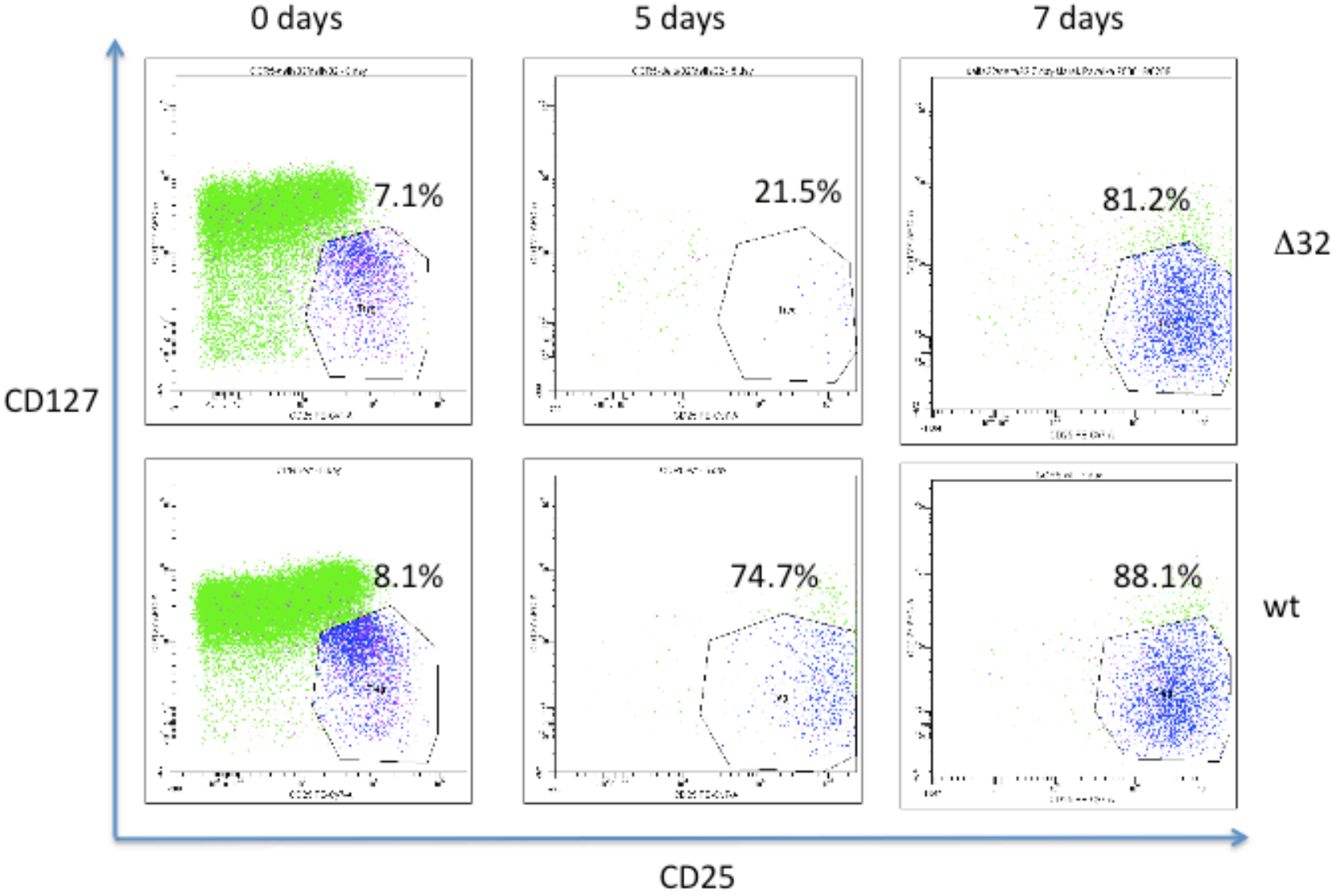
The sampling locations are marked by black points. The interpolation is masked in regions where data are unavailable.

DOI: 10.1371/journal.pbio.0030339.g001

Peripheral CD4^{+} T cells have equivalent levels of Tregs in persons homozygous for the CCR5 variant Δ32



Tregs homozygous for the CCR5 gene variant Δ32 can be expanded *in vitro* tenfold after CD3/CD28 activation in the presence of exogenously added recombinant IL-2



The concept of allogeneic stem cell transplantation of the cells resistant to HIV

using a regimen to improve and modulate the function of regulatory $CD4^+$ T cells (Tregs) favors suppressing graft-versus-host disease (GvHD) while retaining the benefits of graft-versus-leukemia (GvL) effect

- In order to enable hematopoetic stem cell (HSC) transplantation of the cells resistant to HIV we intend to create database of bone marrow (BM) donors as well as umbilical cord blood cells (UCB) homozygous for CCR5Δ32 mutation.
- Secondly, based on detailed knowledge of cyclic adenosine monophosphate (cAMP)-mediated suppression of alloreactive Tcons using Tregs harboring Δ32 mutation we will strive to improve Treg immunosuppression and optimize capacity of the HIV glycoprotein (gp) 120 to ameliorate GvHD.
- For umbilical cord blood cells (UCB) homozygous for CCR5Δ32 mutation we will utilize prostaglandin E_2 (PGE_2)-mediated mechanisms to facilitate allogeneic transplantation and/or downregulate CCR5 expression. Key advantage of this process is less stringent requirements for HLA matching and better engraftment of the cells resistant to HIV.