Meeting Abstract

Cell

# In the PBMC of HIV-Infected Patients, CD8 T Cells Form Conjugates and Kill Latently Infected CD4 T Cells

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The PBMC of untreated HIV-infected patients contain HIV-specific CD8 T cells and their potential targets, CD4 T cells latently infected by HIV. We found that CD8 T cells express perforin and form conjugates with autologous CD4 T cells during both the acute and the chronic phases of HIV infection. We confirmed HIV infection of the conjugated CD4 T cells by in situ PCR. We demonstrated apoptosis of the CD4 T cells in the conjugates using continuous live-cell imaging and further observed and measured it using the Annexin V Fitc Apoptosis Detection Kit. We propose that CD4 T cell annihilation in HIV-infected patients results from the interaction of CD8 T cells with latently HIV-infected CD4 T cells. We suggest the presence of equilibrium between the CTL and the virus, mediated by the HIV Nef protein. The CTL, following Nef inhibition, may eliminate the latently infected CD4 T cell reservoir, curing HIV/AIDS.

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Meeting Abstract

# Interaction of the $XR_{108}$ with $D_{125}$ in HIV-1 Nef Is Important for Oligomeric Association and MHC-I Downregulation

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HIV-1 Nef (27 kD) enforces diverse functions through interactions of specific motifs with various host proteins and apparent adoption of different oligomeric states. Our reported sequences of HIV-1 nef from Indian patients contain a rare deletion of the proteolytic cleavage site that apparently helps in dissociation of Nef from a membrane-associated state to the cytosol. We demonstrate that 22 residues exhibit entropy through structural analysis involving the dimeric, trimeric, and tetramer HIV-Nef associations. Analogous to our earlier reported dimer-tetramer transition in Nef, variations in the relative spatial disposition of Nef subunits around the salt bridge interactions involving XR108 with D125 also exist in dimer-trimer transition. The oligomeric changes affect the accessibility of different surface residues/interaction motifs. This predictably impacts Nef functions by modulating/blocking its respective interactions with host-protein partners. The analysis is supported by our studies involving the disruption of the interactions of D125 with XR108 by a D125A mutation.

Meeting Abstract

Cell

# Dialysis Purification of Integrase-DNA Complexes Provides High-Resolution Atomic Force Microscopy Images: Dimeric Recombinant HIV-1 Integrase Binding and Specific Looping on DNA

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It remains difficult to obtain high-resolution atomic force microscopy images of HIV-1 integrase bound to DNA in a dimeric or tetrameric fashion. We therefore constructed specific target DNAs to assess HIV-1 integrase binding and purified the complex by dialysis prior to analysis. Our resulting atomic force microscopy analyses indicated precise size of binding human immunodeficiency virus type 1 (HIV-1) recombinant integrase in a tetrameric manner, inducing formation of a loop-like or figure-eight-like secondary structure in the target DNA. Our findings regarding the target DNA secondary structure provide new insights into the intermediate states of retroviral integration.

Cell

Meeting Abstract

# Construction of a New Recombinant Fowlpox Virus Shuttle Vector and Its Application in HIV Vaccine Research

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Fowlpox virus (FPV) has potential application in gene expression, vaccine, and gene therapy researches because of its many advantages, especially broad cloning capacity and abortive replication in mammalian cells. However, there are still some problems in current FPV vectors, e.g., low recombinant and expression efficiency and longer screening cycle, etc. In the present study, based on the genome information of FPV, we selected five nonessential genes as homologous recombination sites. Subsequently, the shuttle vectors with triple-gene expression cassette were designed, constructed, and evaluated with fluorescent proteins EGFP, BFP, and RFP. Recombinant virus can highly and stably express at least three genes independently without interaction among them. Then, rFPV HIV vaccine containing HIV-1 multi-epitopes, CpG motif, and CTB was constructed and screened, and the reporter gene was deleted by Cre-loxp system, whose immunogenicity was evaluated by animal models. This study provides a solid foundation for the development of a new HIV-1 candidate vaccine.

Meeting Abstract

Cell

# Construction, Immunogenicity, and Safety Analysis of an HIV-1 Candidate Vaccine Based on an Attenuated Vaccinia Virus

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AIDS caused by HIV has been an important public health problem, and no effective prophylactic or therapeutic vaccine against HIV-1 in humans is currently available. In the present study, based on an attenuated vaccinia virus constructed previously, an HIV-1 candidate vaccine rVV containing HIV-1 multi-epitopes, CpG motif, and CTB gene was constructed successfully by a vaccinia virus shuttle vector, and HIV-1 antigens were highly and stably expressed in mammalian cells infected by rVV. Subsequently, the immunogenicity and safety were evaluated by animal models. Results indicated that the HIV-1 candidate vaccine can induce cellular and humoral immune responses and can elicit certain levels of immunological memory against HIV-1. Additionally, the virulence of rVV was attenuated in vivo of mice compared with wild-type strain and has a certain safety. This paper provides a solid foundation for further studying of HIV candidate vaccine.

Cell

Meeting Abstract

# Antibody-Mediated Depletion of Cell Subsets Carrying Persistent Virus in SHIV-Infected Macaques

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Antibodies can be used to eliminate memory CD4 T cells and/or other cells carrying persistent provirus. Using SHIV-infected pigtailed macaques, aviremic for >6 months, viremia could be elicited by challenge with anti-CD8 mAb. Challenge also boosted Ab responses to Env. We examined the immunologic and virologic consequences of treating macaques with anti-CD45RO, anti-CD4, or both. Subset depletion was monitored by FACS in PBMC, LN, and gut; antiviral effects by measuring provirus and postchallenge viremia; and by measuring Ab responses to trimeric HIV gp140. Complete depletion was not obtained at all anatomic sites. Anti-CD4 plus anti-CD45RO reduced and delayed postchallenge viremia and eliminated circulating provirus during CD4 depletion. We also targeted activated macrophages, with potential effects. Subset depletion did not decrease ''boosting'' of the anti-Env humoral response elicited by challenge. Ab-mediated depletion of memory subsets may be used to deplete persistent viral reservoirs and yet may have limited immunological consequences.

Meeting Abstract

Cell

# Tenofovir Treatment during Pregnancy in Rats: Effects on Offspring

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*Presenting author

Several guidelines advise caution in the use of Tenofovir (TDF) during pregnancy. Aim of this study: to evaluate the occurrence of systemic and renal abnormalities in fetuses exposed to TDF. Methods: Female Wistar rats received standard diet with or without addition of TDF (100 mg/kg diet) 1 week before mating and during pregnancy (n = 3 for each group). Control (n = 5) and TDF (n = 9) offspring were followed up until 6 months old. Results: TDF offspring showed lower birth weight (6.19 ± 0.06 g versus control 6.56 ± 0.07 g; $p < 0.001$). Blood pressure increased after second month in TDF and remained elevated until 6 months (135 ± 3 versus 118 ± 2 mm Hg; $p < 0.005$). TDF showed lower renal excretion of sodium - FENa (0.86 ± 0.06 versus 1.23 ± 0.19%; $p < 0.05$) associated with significant increase in renal expression of angiotensin II (129 ± 9 versus 101 ± 1%; $p < 0.05$), AT1 receptor (173 ± 23 versus 100 ± 11%; $p < 0.05$) and Na-K-2Cl cotransporter (148 ± 6 versus 100 ± 7%; $p < 0.001$). Conclusions: Maternal exposure to TDF during pregnancy results in salt-sensitive hypertension in offspring.

# Protective Effect of N-Acetylcysteine on Chronic Tenofovir Nephrotoxicity in Rats

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Tenofovir (TDF) is associated with chronic kidney disease and increased oxidative stress. The aim of this study was to evaluate the effect of the antioxidant N-acetylcysteine (NAC) on TDF nephrotoxicity. Three groups of male rats were studied: (1) control (C; n = 8); (2) TDF (50 mg/kg diet; n = 10); and (3) TDF 50 mg/kg diet + NAC 600 mg/l drinking water; n = 10. Clearance studies were performed after 4 months of treatment. TDF decreased glomerular filtration rate (GFR, ml/min/100 gBW) (C = 0.63 ± 0.04 versus TDF = 0.40 ± 0.03; $p < 0.001$), increased blood pressure (BP, mmHg) (C = 126 ± 5 versus TDF = 139 ± 3; $p < 0.05$), and angiotensin II expression in renal tissue (C = 100 ± 9 versus TDF = 174 ± 22%; $p < 0.05$). These effects were associated with an increased oxidative stress evaluated by serum thiobarbituric-acid-reactive substances (TBARS, nM/ml) (C = 1.92 ± 0.18 versus TDF = 5.04 ± 0.34; $p < 0.001$) and decreased serum gluthatione, an endogenous antioxidant (μM/ml) (C = 2.70 ± 0.15 versus TDF = 2.18 ± 0.07; $p < 0.01$). NAC reversed all of these effects (GFR = 0.59 ± 0.04; BP = 114 ± 5; angiotensin II expression = 95 ± 5%; TBARS = 3.51 ± 0.16; gluthatione = 2.68 ± 0.08; $p < 0.01$ versus TDF). These findings have clinical implications for protection against chronic TDF nephrotoxicity.

Meeting Abstract

Cell

# Neutralizing and Nonneutralizing Antibody Binding to Soluble Cleaved and Uncleaved HIV-1 Env Trimers and Protomers

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Soluble Env that mimics functional trimers should ideally bind only neutralizing antibodies. Antibody binding to BG505 (clade A) SOSIP.664 Env was studied by surface plasmon resonance. Proteolytically processed trimers were compared with uncleaved ones, gp140 protomers, and gp120 monomers. The antibodies PGV04, VRC01, PG9, PG16, PGT121, PGT123, PGT135, PGT145, and 2G12 neutralized BG505 and bound well to cleaved trimers; b6, b12, 14e, and F240 did not neutralize and bound negligibly. Conversely, b6 and b12 bound well, and PG9, PG16, and PGT145 bound poorly to uncleaved trimers, protomers, and gp120. VRC01 and 2G12 bound similarly to trimers and protomers. Kinetics, affinity, and stoichiometry for sCD4, PG9, PG16, PGT121, PGT123, PGT128, and 2G12 agreed, where applicable, with calorimetric data and electron micrographs; the latter also differentiated morphologically between cleaved and uncleaved trimers. The antigenicity and structural integrity of cleaved BG505 SOSIP.664 trimers may favor elicitation of broadly neutralizing antibodies through vaccination.

Cell

Meeting Abstract

# Potent Elimination of HIV from Infected Cells In Vitro by IgG-Conjugated Enfuvirtide

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Enfuvirtide (T20) is the only marketed HIV fusion inhibitor, requiring a twice-daily subcutaneous injection. We have site-specifically tethered a dimerized T20 at each of the carboxyl termini of either the heavy or light chain of a humanized IgG, resulting in a class of novel anti-HIV agents with four molecules of T20 that potently neutralize primary isolates (both R5-tropic and X4-tropic), as well as T-cell-adapted strains of HIV-1 in vitro, with $EC_{50}$ values in the subnanomolar range, which are 10- to 100-fold lower than enfuvirtide and are attainable whether or not the constitutive antibody targets HIV-1. The potential of such conjugates to purge latently infected cells was also demonstrated in a cell-to-cell viral inhibition assay following viral activation with 100 nM SAHA. Thus, judiciously attaching multiple copies of T20 on a full IgG should overcome the limitations of enfuvirtide and may lead to new therapies for eradicating latently infected cells.

Meeting Abstract

Cell

# Regulation of HIV-1 Gene Expression and Replication by Heat-Shock Proteins

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HIV-1 hijacks the cellular machinery for accomplishing its life cycle leading to significant modulation in host cell gene expression. Induction of heat-shock proteins (HSPs) is one such early event observed after virus infection. Kinetic expression profiling studies in HIV-1-infected T cells show that expression of most HSPs is differentially modulated during the course of infection. Our results also show that HSP70 is induced at the transcription level in a Nef-dependent manner. We have further analyzed the role of the HSP70 cochaperone HSP70-binding protein 1 (HspBP1) during HIV-1 infection. Silencing of HspBP1 leads to increased virus production, whereas its overexpression inhibits viral replication. Our results indicate that there could be a co-operative interaction between HSP70 and HspBP1, which can inhibit HSP40-mediated increase in HIV-1 gene expression. Thus, our results suggest that the intricate interplay between different HSPs contributes significantly toward the regulation of HIV-1 gene expression and replication.

Cell

Meeting Abstract

# Novel Triterpenes Activate Latent Human Immunodeficiency Virus Expression

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Viral reservoirs established during HIV infection remain unaffected by current treatment regimens. Therapeutic targeting of viral latency may provide a solution to eradicating HIV-1 in infected individuals. Triterpenoids isolated from *Ocimum labiatum* (*Lamiaceae*) was investigated for the induction of HIV-1 replication in latently infected U1 cells. Viral expression was detected by measuring the HIV-1 p24 antigen released in culture media. Phorbol 12-myristate 13-acetate (PMA), a known HIV-1 inducer in U1 cells, was included as a control. The isolated triterpene isomers significantly ($p < 0.05$) induced HIV-1 expression in U1 cells at a concentration that was not toxic to the cells. The triterpenes further induced viral expression synergistically with PMA. These findings suggest the potential of the isolated compounds for development into drug candidates to be used in concert with existing HIV drugs, whereby the terpenes activate latent viral expression and HAART reduces or prevents viral multiplication.

Meeting Abstract

Cell

# Hexameric HIV-1 Envelope Glycoprotein Immune Complexes as Vaccine Immunogens

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Current envelope glycoprotein (Env) subunit vaccines induce nonprotective immune responses. We explored the adjuvant activity of the Fc domain of antibodies by generating artificial Env immune complexes (Env-ICs). Conventional ICs (antibodies in complex with their cognate antigen) enhance uptake by dendritic cells (DCs), DC activation, and antigen presentation for T help and stimulate antibody affinity maturation and isotype switching by B cells. We fused the Fc region of human IgG1 to trimeric BG505-SOSIP-664 gp140. The resulting BG505 SOSIP-Fc protein formed predominantly hexamers consisting of two Env trimers and three Fc dimers. Hexameric BG505 SOSIP-Fc was antigenically identical to BG505 SOSIP.664 and interacted efficiently with quaternary structure-dependent antibodies PG16 and PGT145. Env-ICs were more efficiently captured by DCs and activated T helper cells specific for gp120 more potently than conventional Env antibody ICs or Env alone. Mouse immunization experiments with Env-ICs containing mouse IgG1, IgG2a, IgG2b, and IgG3 Fc tail are ongoing.

Cell

Meeting Abstract

# Immunosilencing of a Highly Immunogenic Multimerization Domain Used in Therapeutic Proteins and Vaccines

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Many therapeutic proteins and protein-based vaccines contain heterologous multimerization domains that can potentially induce adverse antibody responses that can affect their efficacy. The widely used GCN4-based isoleucine zipper (IZ) trimerization domain induced extremely potent anti-IZ antibody responses in rabbits when fused to an HIV-1 envelope glycoprotein (Env) vaccine. To dampen these responses, we added four N-linked glycans (N4) to the IZ domain and vaccinated rabbits with IZ and IZN4, both fused to Env as well as to influenza hemagglutinin (HA). Glycosylation of IZN4 did not affect the antibody titers against Env or HA, but the antibody responses against the IZ domain were dramatically reduced. Immunosilencing of multimerization domains by adding glycans might be generally relevant for the design of protein vaccines and therapeutic proteins such as HIV-1 Env, influenza HA, and CD40 ligand that depend on quaternary structure for their immunogenicity and/or biological activity.

Meeting Abstract

Cell

# A Small-Molecule HIV Entry Inhibitor Selectively Inhibits Cotranslational Translocation of CD4 into the Endoplasmic Reticulum

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Regulating CD4, a crucial receptor for HIV, can be an important target for antiviral intervention. We have identified the small-molecule cyclotriazadisulfonamide (CADA) that downmodulates CD4 expression in immune cells and prevents HIV entry. A correlation between CD4 downmodulation and anti-HIV activity was also observed for new unsymmetrical CADA analogs bearing a side arm with high electron density. From our investigation on the mechanism of action, we can conclude that CADA requires the N-terminal signal sequence of the CD4 protein to inhibit its biosynthesis. Cell-free in vitro translation experiments with CD4/preprolactin chimera revealed that CADA prevents the translocation of CD4 across the ER membrane in a signal-sequence-dependent manner. Residues within the hydrophobic segment of the CD4 signal peptide were identified as critical for compound sensitivity. Our findings demonstrate that cell-permeable compounds can selectively inhibit protein biosynthesis and highlight cotranslational translocation at the ER as a potential target for antiviral therapy.

Cell

Meeting Abstract

# The Disulfide Bond Architecture of the HIV-1 Envelope Glycoprotein V1V2 Domain Affects Trimer formation

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The envelope glycoprotein (Env) of HIV-1 is the target of broadly neutralizing antibodies (bNAbs) that have the potential to provide protective immunity. Shielding of bNAb epitopes by highly flexible variable loops on gp120 limits the induction of bNAbs. To reduce V1V2 flexibility, we designed three double-cysteine mutants (E153C-R/K178C, L/I154C-Y177C, and K155C-F176C) that were characterized in the context of BG505 SOSIP.664 gp140, a recombinant mimic of the native Env spike, as well as infectious LAI virus. Surprisingly, all three mutants showed dramatically decreased trimer formation (BG505 SOSIP.664) and completely abrogated infectivity (LAI virus). After prolonged culturing of the virus, we identified a secondary site reversion G152E immediately adjacent to the introduced disulfide bond (E153C-R/K178C), which partially restored infectivity of LAI virus and trimer formation of the corresponding BG505 SOSIP.664 gp140 protein. Our findings point at a crucial role of the V1V2 disulfide bond architecture for Env trimer formation.

Meeting Abstract

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# A Next-Generation Cleaved, Soluble HIV-1 Env Trimer, BG505 SOSIP.664 gp140, Expresses Multiple Epitopes for Broadly Neutralizing, but Not Nonneutralizing, Antibodies

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A desirable but as yet unachieved property of an HIV-1 vaccine candidate is the ability to induce broadly neutralizing antibodies (bNAbs). One approach is to create trimeric mimics of the native envelope glycoprotein (Env) spike that expose as many bNAb epitopes as possible while occluding those for nonneutralizing antibodies (non-NAbs). We generated a soluble, cleaved (SOSIP.664) gp140 trimer based on the subtype A strain BG505. Tagging the trimer allowed its oriented immobilization on ELISA plates and detailed antigenic characterization. Virtually all bNAbs against multiple epitope clusters were highly trimer reactive, whereas most non-NAbs did not react, even when their epitopes were present on simpler forms of Env. Overall, there was an excellent correlation between mAb binding to the SOSIP.664 trimer by ELISA and mAb neutralization of the parental virus. Thus, BG505 SOSIP.664 gp140 is a close antigenic mimic of the virus-associated Env spike and might be valuable as an immunogen.

Cell

Meeting Abstract

# Restricted Endogenous IRF-1 Expression Limits HIV-1 LTR Transactivation while Temporally Shortening Host Immune Activation

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Elevated expression of host interferon regulatory factor-1 (IRF-1) is required for the early transactivation of HIV-LTR. Low levels of IRF-1, also a key immunoregulatory factor, associate with natural resistance to HIV infection. This study examined the functional importance of restricting endogenous IRF-1 expression in limiting HIV-1 replication and regulating host antiviral responses. Reducing endogenous IRF-1 expression in ex vivo peripheral $CD4^+$ T cells and monocytes by $\sim$30%–50% with siRNA resulted in $\geq$90% reduction in the transactivation of HIV-LTR and, hence, decreases in viral gene expression and viral replication. Moreover, reduced IRF-1 level posed no effects on the HIV-1-elicited activation of host IRF-1-regulated immunologic genes but significantly lessened the duration of host immunologic responses. These data suggest that suboptimal IRF-1 levels could effectively limit productive HIV infection but remain sufficient for activating a robust yet transient immune response, limiting the spread of HIV-1 infection and the undesirable immune activation.

Meeting Abstract

Cell

# Induction of Autophagy by ASP, an Unusual HIV-1 Protein Translated from an Antisense Transcript

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*Presenting author

It is well known that HIV-1 proteins are translated from a single transcript in an unspliced manner or following splicing. In 1988, a study suggested the existence of a new HIV-1 protein that had the originality of being translated from an antisense transcript. We now know that this antisense transcript, which is unspliced and polyadenylated, encodes for an unusual protein named ASP (antisense protein).Our results demonstrate that ASP is a cytoplasmic punctuated protein associated with autophagosomes that can induce autophagy, an important mechanism for HIV-1 replication in macrophages. In conclusion, further study of ASP is an important issue for the understanding of HIV-1 and its pathogenesis.

Cell

Meeting Abstract

# A Novel Tat Inhibitor Prevents HIV-1 Reactivation

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The HIV Tat protein, a potent activator of HIV gene expression, is essential for integrated viral genome expression and represents a potential antiviral target. Tat binds the 5′ terminal region of HIV mRNA's stem-bulge-loop structure, the transactivation-responsive (TAR) element to activate transcription. We found that didehydro-Cortistatin A (dCA), an analog of a natural steroidal alkaloid from a marine sponge, inhibits Tat-mediated transactivation of the integrated provirus by binding specifically to the TAR-binding domain of Tat. Working at subnanomolar concentrations, dCA reduces Tat-mediated transcriptional initiation/elongation from the viral promoter to inhibit HIV-1 replication in acutely and chronically infected cells. Furthermore, in vitro dCA abrogates low-level virus replication from primary cells isolated from patients undergoing antiretroviral therapy (ART) and prevents reactivation upon homeostatic stimulation with cytokines such as IL15 and antigenic stimulation by CD3/CD28. These results define dCA as a novel anti-HIV drug that could decrease residual viremia during ART.

Meeting Abstract

Cell

# Modeling a Cure for HIV in Nonhuman Primates

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In 2009, a single HIV-positive patient was cured of HIV following allergenic transplantation of hematopoietic stem cells (HSCs) from a *CCR5*$^{-/-}$ donor. However, the utility of this approach is severely limited due to the difficulties in finding *CCR5*$^{-/-}$ matched donors. We are interested in modeling factors that led to cure and extending this treatment to the autologous setting using gene-modified and HIV-resistant stem cells. We have recently described a model of HIV infection on antiretroviral therapy (ART) in the pigtailed macaque using the env-SHIV1157ipd3N4, followed by three-drug ART. In addition, we showed that transplantation with mC46-expressing HSCs leads to positive selection of gene-modified CD4$^{+}$ T cells and drastic reduction in viremia following SHIV challenge. Furthermore, our results demonstrate that only partial engraftment of modified HSCs may lead to protection. Our studies position us to evaluate gene-therapy-based functional cure strategies in a clinically relevant model of HIV infection.

Cell

Meeting Abstract

# MPER Peptides with Chemical Modifications Retain Monoclonal Antibody Binding and Generate High Titer Antisera in Rabbit Immunizations

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Broadly neutralizing monoclonal antibodies, 2F5 and 4E10, cross-react with their respective gp41 epitopes and phospholipids, which may be driven by the phosphate head group. To exploit this potential interaction, we investigated whether anionic moieties on residues within the MPER sequence would generate high titer antisera. We synthesized a series of chemically modified peptides that span the MPER. Serine, threonine, and tyrosine residues in the peptides were modified with sulfonates, phosphates, or nitrates and were presented in liposomes for rabbit immunizations. All immunizations resulted in high-titer antisera (>$10^5$ reciprocal titer) directed toward the modified and unmodified immunogens. Sera with strong anti-gp140 titers were purified and possess a higher affinity toward the MPER when compared to 2F5 and 4E10. Neutralization assays with sera and purified antibodies are currently ongoing. We conclude that anionic modifications induce strong anti-immunogen antibodies, suggesting a role for chemical modifications of epitopes in HIV and other disease models.

Meeting Abstract

Cell

# Regulation of IRF-1 Responses and Susceptibility to HIV Infection

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Interferon regulatory factor 1 (IRF-1), a transcription regulator of antiviral responses, is upregulated and essential for the transactivation of HIV-LTR during early HIV infection. However, little is known of how IRF-1 expression is regulated in nontransformed cells and in response to HIV infection. We showed earlier that HIV-exposed seronegative individuals (HESN) have reduced baseline IRF-1 expression and robust yet transient IRF-1 responses, perhaps epigenetically regulated at the promoter. Here, we identified a highly conserved, enhancer-like region (−6220 to −6270 of IRF-1) that comprises binding motifs for IRF-1/NF-κB/p300/STAT1. This enhancer-like region was highly acetylated and was bound by IRF-1 following exogenous IFN-γ stimulation in healthy HIV-susceptible controls but lacked both in HESN, where a transient upregulation of IRF-1 was observed. Further evidence suggests that this acetylated, IRF-1 bound enhancer-like region looped back to IRF-1 promoter, supporting the continuous increases of IRF-1 expression in HIV-susceptible controls; hence, it may play a critical role in susceptibility to HIV-infection.

Cell

Meeting Abstract

# Inhibiting the Tyrosine Kinase c-Src Restricts HIV-1 Integration in CD4$^+$ T-Lymphocytes

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HIV-1 infection of CD4$^+$ T-lymphocytes activates the kinase c-Src within minutes of infection; however, a role for c-Src activation by HIV-1 remains to be determined. We infected activated CD4$^+$ T cells with replication-deficient HXB2(X4) or JR-FL(R5) luciferase reporter viruses in the presence or absence of c-Src inhibitors or siRNAs to determine the effect on HIV-1 infection. Four different c-Src inhibitors caused a marked decrease in luciferase activity post-HXB2 infection. siRNA knockdown targeting c-Src also reduced luciferase activity of HXB2 or JR-FL infection, and real-time qPCR showed inhibition of viral integration and increased 2-LTR circle formation. Interestingly, this coincided with an increase in late reverse transcripts of HIV-1. Reduced integration, coupled with accumulating late reverse transcripts, suggests that c-Src may exert its role during pre-integration complex formation or nuclear import of viral cDNA. Kinase inhibition of c-Src may provide a novel means to inhibit HIV-1 infection at the level of pre-integration.

Meeting Abstract

Cell

# Mobilizing Systemic Immune Responses to the Genital Mucosal Compartments by Toll-like Receptor 9-based Microbicides

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Vaccine strategies that can elicit immunity at mucosal surfaces would protect against many infectious pathogens. In this study, we investigate a strategy of first generating a systemic immune response through conventional vaccination (Prime) and then localizing immune responses to the female genital mucosa through topical application of CpG oligonucleotides (ODNs) (Pull). We demonstrate the prophylactic efficacy of the Prime-Pull strategy in a murine model of herpes simplex virus 2 (HSV-2) challenge. Mice receiving the Prime-Pull vaccination survived a lethal HSV-2 challenge at least 8 weeks post-pull compared to mice receiving only the Prime or the Pull. In addition, the surviving mice were protected from a second lethal dose of HSV-2 challenge. This strategy presents a simple methodology that can augment current successful systemic vaccination strategies for protection at mucosal surfaces against a variety of infectious pathogens.

Cell

Meeting Abstract

# Boosting Normal Homeostasis May Expedite the Elimination of HIV-Infected Memory T Cells from Patients on ART

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During effective ART, provirus-containing $CD4^+$ memory T cells are maintained, in part, through homeostatic self-renewal and, in part, by proliferation during recurring immune-response-like proliferation bursts and possibly also by low-level de novo infection. Existing memory cells specifically compete with naive cells and newly emerging memory cells for niches defined by antigen-presenting cells. In the absence of significant de novo infection, uninfected cells have competitive advantage over infected cells, and less-differentiated cells over more differentiated ones. Therefore, once the naive compartment has sufficiently recovered, inducing accelerated polyclonal turnover of $CD4^+$ T cells might boost replacement of infected cells with uninfected cells. Two strategies might be implemented concomitantly under maximally suppressive ART. First, controlled, time-structured introduction of agents that partially deplete $CD4^+$ T cells while maintaining the minimal diversity of Tregs required to prevent autoimmunity. Second, targeting adjuvants to lymphoid tissues to temporarily overcome control by Tregs and by activation-threshold tuning of the desired homeostatic responses.