the steeper slopes reflected later time or lower γ. Adsorption at constant γ and to preexisting films showed that the steeper slope depended on γ rather than time, indicating that the acceleration resulted from changes in the film rather than in the adsorbing material. Compositional studies tested how removing cholesterol, the anionic phospholipids, and the hydrophobic proteins affected the late acceleration. Only the absence of the proteins eliminated the late adsorption. Vesicles of phospholipid with dioleoyl phosphatidylethanolamine, however, which, like the surfactant proteins, promotes fusion between vesicles, also demonstrated the accelerated late adsorption. These results suggest that the late acceleration occurs when vesicles that adsorb by fusion with the nascent film can interact more readily with a more densely packed monolayer. (Small angle x-ray diffraction studies conducted at the Stanford Synchrotron Radiation Lightsource.)

3431-Pos Board B536

Dihydrosphingomyelin Impairs HIV-1 infection by Rigidifying Liquid-Ordered Membrane Domains

Catarina R. Vieira, Jose M. Munoz-Olaya, Jesús Sot, Sonia Jiménez-Baranda, Nuria Izquierdo-Useros, José-Luis Abad, Beatriz Apellániz, Rafael Delgado, Javier Martínez-Picado, Alicia Alonso, Josefina Casas, José L. Nieva, Gemma Fabriás, **Félix M. Goñi**.

The lateral organization of lipids in cell membranes is thought to regulate numerous cell processes. Most studies focus on the coexistence of two fluid phases, the liquid crystalline (ld) and the liquid-ordered (lo); the putative presence of gel domains (so) is not usually taken into account. We show that in phospholipid:sphingolipid:cholesterol mixtures, in which sphingomyelin (SM) promoted fluid lo domains, dihydrosphingomyelin (DHSM) tended to form rigid domains. Genetic and pharmacological blockade of the dihydroceramide desaturase (Des1), which replaced SM with DHSM in cultured cells, inhibited cell infection by replication-competent and deficient HIV-1. Increased DHSM levels gave rise to more rigid membranes, resistant to the insertion of the gp41 fusion peptide, thus inhibiting viral-cell membrane fusion. These results clarify the function of dihydrosphingolipids in biological membranes and identify Des1 as a potential target in HIV-1 infection.

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Cholesterol and Low Temperature Enhance Fusion of Vesicles to a Planar Bilayer

David E. Lee, Matthew G. Lew, Reed A. Doxey, Dixon J. Woodbury.

Lipid composition plays an important role in fusion of vesicles to membranes, an essential process for exocytosis. Lipid head group, tail structure, and sterol content all impact the complex phase behavior of membranes. To determine the effect of lipids on fusion rate, we utilized the nystatin/ergosterol (nys/erg) fusion assay and stimulated fusion with an osmotic gradient. Using PE/PC planar membranes with increasing concentrations of cholesterol, and keeping vesicle lipids constant (PE/PC/PS/ERG), we observed increasing fusion rates. We also observed that temperature affects fusion rates by forming DPPC membranes on 5ul glass capillary pipets. Planar membranes were painted with concentrated DPPC in decane. Significantly different fusion rates were observed at temperatures above and below the DPPC transition temperature (Tt) of 41C. Decreasing the temperature below Tt increased the fusion rate, while increasing the temperature above Tt reduced the fusion rate. These data are consistent with the hypothesis that vesicle fusion with a membrane is suppressed in a liquid disordered lipid phase, and shows how membrane fusion can be affected by lipid behavior.

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Cholesterol as a Modulator of the HIV-1 gp41 Fusion Domain's Function

Andrey Ivankin, David Gidalevitz.

The early steps in HIV-1 infection of cells involve fusion of the envelope with the host cell membrane. The N-terminal fusion domain of the viral envelope glycoprotein 41 subunit is widely accepted to play a key role in the fusion process facilitating membrane anchoring, destabilization, and bending. Ability of the fusion domain to perform these essential functions depends strongly on the structural and mechanical properties of the host cell membrane, which are defined by the lipid composition and cholesterol content. Here we present results of an X-ray study aimed at the understanding the effect of cholesterol concentration in model lipid membranes on the activity of the viral fusion domain. Lipid monolayers at the air-liquid interface composed of DPPC and cholesterol were used to model an approximate environment where the fusion domain comes into contact with the host cell membrane. The electron density profiles across the films, derived from X-ray reflectivity data, demonstrate that the fusion domain penetrates into all DPPC/cholesterol monolayers. The depth of membrane insertion and orientation/conformation of the fusion domain within the film, as well as lipid-to-fusion peptide ratio, depend strongly on the membrane cholesterol concentration. Distinct insertion modes also suggest that the fusion domain-induced membrane curvature is considerably different in bilayers with low and high cholesterol content. Finally, using grazing incidence X-ray diffraction we have demonstrated that the viral fusion domain possesses limited membrane destabilizing effect and is incapable to degrade the gel phase in the DPPC/cholesterol films.

3434-Pos Board B539

Destabilization of Highly Rigid Bilayers Enriched in Cholesterol by the Membrane-Proximal External Region of HIV-1 gp41

Beatriz Apellaniz, Andrey Ivankin, David Gidalevitz, **Jose L. Nieva**.

Current models for HIV fusion incorporate the induction of curvature stress and/or membrane defects by the membrane-proximal external region (MPER) of the envelope glycoprotein 41 subunit. The enrichment in aromatic residues predicts a highly favorable membrane-bound state for MPER, which would insert into the interface of the viral membrane external monolayer. However, the high levels of cholesterol found in the lipid envelope (Cholesterol-to-phospholipid mole ratio: ≈ 1) suggest that MPER insertion will be hampered in functional virions. Here, we have varied the membrane composition in order to test systematically bilayer area compressibility modulus (K_a) and monolayer spontaneous curvature (R_o) effects on binding and pore opening by MPER-derived peptides. In addition, we have assessed the degree of exposure to solvent, based on the reactivity to anti-MPER antibody. Finally, using grazing incidence X-ray diffraction (GIXD) and specular X-ray reflectivity (XR) we have studied the interactions of the MPER peptides with phospholipid monolayers containing low and high levels of Cholesterol. Our results support a role for MPER in destabilizing the highly rigid envelope, and suggest that the establishment of favorable MPER-cholesterol interactions may help overcoming the restrictions to the insertion process.

3435-Pos Board B540

Entry Pathways of an Avian Virus into Cells Expressing Transmembrane and GPI-Anchored Receptor Isoforms

Naveen Jha, Erik Martin, Olga Latinovic, Mariana Marin, Gennadiy Novitskiy, Kosuke Miyauchi, **Gregory Melikian**.

Avian Sarcoma and Leukosis Virus (ASLV) entry into cells proceeds through two major steps -priming by cognate receptors on the cell surface followed by low pH-induced fusion with endosomes. Subtype A viruses are able to utilize two naturally occurring isoforms of the TVA receptor for infection, the GPI-anchored (TVA800) and the transmembrane (TVA950) receptors, which are localized to different membrane domains. We monitored the consecutive steps of ASLV entry into cells expressing these receptor isoforms, including endocytosis, delivery into acidic compartments, and fusion with endosomes. Cells expressing TVA950 internalized ASLV much faster than those expressing the alternative receptor. In both cell lines, fusion occurred shortly after internalization, suggesting that virus uptake was the rate-limiting step of entry. We found that ASLV fusion with endosomes proceeded through two relatively long-lived intermediates, a hemifusion-like intermediate and a small fusion pore. The lifetime of these intermediates was dependant on TVA receptor isoforms and their expression levels. TVA950 supported more robust fusion intermediates compared to TVA800. These findings are consistent with ASLV trafficking by via TVA950 to endosomal compartments that are more conducive to fusion. Alternatively, transmembrane receptor might more efficiently prime the viral fusion proteins compared to the GPI-anchored receptor. This work has been supported by the NIH AI053668 grant.

3436-Pos Board B541

Trans-Membrane Domain of HIV gp41 Interacts with the Externally Added gp41 Fusion Peptide: TMD-FP Complex Inhibits Model Membrane Fusion

Hirak Chakraborty, David G. Klapper, Barry R. Lentz.

We report the effects of a complex between fusion peptide (FP) and transmembrane domain (TMD) of gp41, that separately promote fusion, on the kinetics of PEG-mediated fusion and on the structure of the bilayer. Circular dichroism (CD) measurements showed that FP in the membrane exists mostly in a beta sheet at L/P ratios 1200/1 to 200/1, while the gp41 TMD displayed ~32% helix, 23% beta, 21% turn and 24% other secondary structure content at L/P of 300/1. Adding FP to membranes containing TMD resulted in a TMD-FP complex with reduced helical content and increased beta and