VIRAL IMMUNITY

Crafty rafting

Lentiviruses, including HIV-1, assemble at the host-cell plasma membrane. Although this multistage process is known to be mediated by viral Gag proteins, the exact nature of the membrane-assembly programme is poorly understood. Reporting in *Proceedings of the National Academy of Sciences* Ono and Freed show that HIV-1 Gag associates with cholesterol-enriched microdomains (rafts) at the plasma membrane, and that this process is crucial for HIV-1 particle formation and release.

The idea that viruses might bud from distinct microdomains in the plasma membrane is based on the finding that the lipid composition of envelope membranes from numerous viruses differs from the host plasma membrane from which they are derived. The HIV-1 lipid bilayer is enriched in sphingolipids and cholesterol. To investigate whether HIV-1 Gag associates with rafts at the plasma membrane, the authors homogenized cells expressing $Pr55^{Gag}$ (the Gag-precursor protein) and isolated rafts as detergent-resistant membranes (DRMs). They found that 50% of membrane-bound $Pr55^{Gag}$ specifically associated with rafts.

To identify the domains of $Pr55^{Gag}$ that are required for DRM association, Ono and Freed characterized the DRM-association of *Gag* mutants. These experiments showed that the N-terminal matrix domain of Gag is required for raft binding, and that sequences downstream of this domain which promote Gag–Gag interactions stabilize raft association.

But is this Gag–raft association physiologically relevant in HIV-1 particle assembly and release? The authors disrupted rafts in HIV-1-infected cells with cholesterol-depleting drugs and showed that this caused impaired HIV-1 release and infectivity. Therefore, the association of Gag with rafts is crucial for efficient HIV-1 assembly and release, and the authors conclude that these findings might permit the development of new strategies to suppress HIV-1 replication *in vivo*.

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References and links

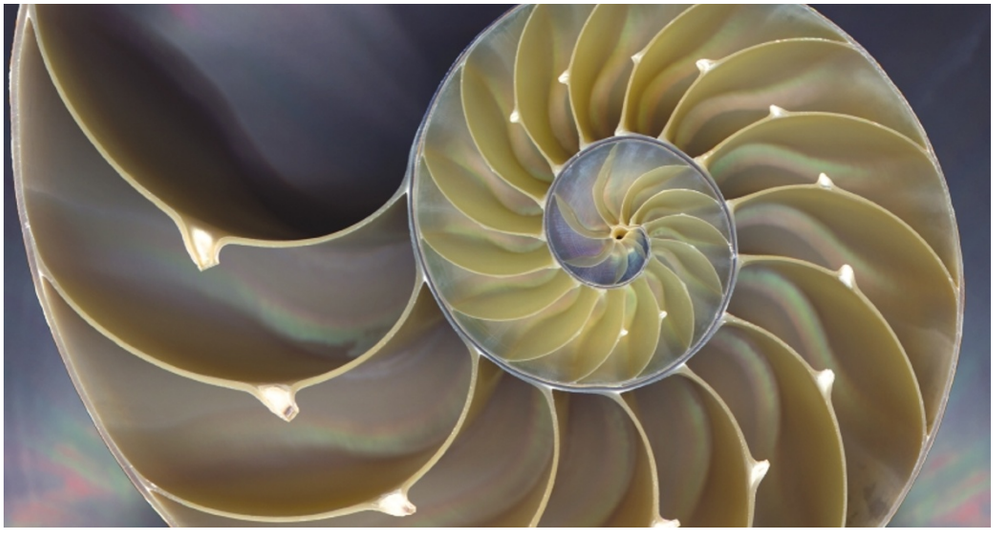
ORIGINAL RESEARCH PAPER Ono, A. & Freed, E. O. Plasma membrane rafts play a critical role in HIV-1 assembly and release. *Proc. Natl Acad. Sci. USA* **98**, 13925–13930 (2001)

GENE THERAPY

Outside-in

One barrier to the successful application of gene therapy is that although many options exist for transfecting cells *in vitro*, these techniques are often not easily adapted for *in vivo* use. This is particularly true for attempts to reverse immunodeficiency in the lung. Alveolar macophages (AMs) are essential for the generation of an effective inflammatory response against pathogens invading the alveolar space. During systemic immunosuppression, the function of these phagocytic cells diminishes, and the risk of opportunistic lung infections increases. Wu and colleagues now describe, in *Proceedings of the National Academy of Sciences*, an approach to reverse alveolar immunodeficiency through transferring the mouse interferon-γ (*Ifnγ*) gene into AMs *ex vivo*, before airway delivery of the genetically modified cells.

The authors used a recombinant retroviral system to produce IFN-γ-expressing retrovirus with which they infected a mouse macrophage cell line, J774A.1. Transduced cells produced significant levels of IFN-γ, whereas none was detected in the culture supernatant of uninfected cells. Next, the IFN-γ-producing macrophages were transferred intratracheally into severe combined immunodeficient (*scid*) mice. IFN-γ was readily detected in the bronchoalveolar lavage (BAL) fluid from these mice, in contrast to mice instilled with macrophages infected with control virus. Although the expression levels of IFN-γ gradually decreased, it was still detectable in BAL fluid 1 month after instillation.



How did the overexpression of IFN-γ in the alveolar spaces of the recipient mice affect immune function? AM function was partially restored, resulting in enhanced MHC class II expression and increased phagocytic activity of these cells, detectable even 14 days after instillation of IFN-γ-producing macrophages. In addition, increased levels of the pro-inflammatory cytokine tumour necrosis factor-α were present in the BAL fluid of recipient mice.

The authors conclude that the airway delivery of genetically engineered macrophages expressing IFN-γ can enhance immune activity in the alveolar spaces of immunodeficient animals, and that this approach might be appplicable for gene therapy of immunocompromised patients who are susceptible to opportunistic lung infections.

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References and links

ORIGINAL RESEARCH PAPER Wu, M. *et al.* Genetically engineered macrophages expressing IFN-γ restore alveolar immune function *in scid* mice. *Proc. Natl Acad. Sci. USA* **98**, 14589–14594 (2001)