or green (streptavidin-Alexa488) fluorochromes, intact molecules appeared yellow due to the proximity of the green and red fluorochromes binding intact TBK1, while cleaved TBK-1 produced two distinct bands, one labeling with the green fluorochrome, and one labeling with the red reagent. This demonstrated that HIV-1 PR could cleave TBK1 into the N-terminal green and C-terminal red fragments in vitro. PR D25N, a mutant of HIV-1 PR lacking proteolytic activity, demonstrated that the active site for viral and kinase proteolysis were the same. To verify that the cleavage of TBK1 by HIV-1 PR could occur intracellularly, HEK293 cells were co-transfected with HIV-1 Gag-Pol (Pol includes the protease) or a protease-negative HIV-1 Gag, with TBK1 in the presence or absence of the protease inhibitor, amprenavir. Gag-Pol, unlike Gag, was able to cleave TBK1 in the absence, but not presence of the inhibitor, indicating that TBK1 cleavage by HIV-1 PR occurs intracellularly.

Through amino acid sequencing, it was found that TBK1 was cleaved by HIV-1 PR between L683 and V684. From these data, TBK1 constructs with mutations at the cleavage site were generated. Western blot analysis of the WT TBK1 vs TBK1 mutants demonstrated that these amino acids were required for PR-mediated cleavage.

These experiments confirmed that HIV-PR cleaves TBK1, however, for this to be responsible for immunosuppression, it was necessary to show that cleaved TBK1 was incapable of activating the IFN-1 pathway. Therefore, HEK293 cells were co-transfected with IFNβ-promoter-Luc and either full length TBK1 or a cleaved TBK1 and monitored for luciferase activity. An increase in luciferase activity with full length TBK1, but not cleaved TBK1, was observed, demonstrating that cleaved TBK1 was unable to activate the IFNβ promoter. To further confirm that PR cleaved and inactivated TBK1, HEK293 cells were co-transfected with IFNβ-promoter-Luc, HIV-1 PR, wild-type (wt) TBK1 or a mutant (mt) TBK1. As predicted, wt, but not mtTBK1, -dependent luciferase activity was inhibited by the presence of HIV-1 PR. To determine whether HIV-1 PR inhibits both TBK1 and IRF3 phosphorylation, HEK293 cells were transfected with HIV-1 PR and TBK1, and a western blot for phosphorylated protein was conducted. In the presence of HIV-1 PR, TBK1 fails to become phosphorylated. Further, immunofluorescence was also used to demonstrate that IRF3 remained localized in the cytoplasm when PR was present.

Protease inhibitors (PIs) are commonly used to treat HIV-1, however due to widespread use, viral proteases have become resistant to these PIs. It was possible that PI-resistant PRs might lack the ability to effectively cleave host's proteins. To determine if this occurred, TBK1 were co-transfected in HEK293 cells with either drug resistant mutants of HIV-1 PR or wtHIV-1 PR , followed by western blot for TBK-1 cleavage. While wtHIV-PR was able to affectively cleave TBK1, the drug resistant mutants did not. Interestingly, mt PRs retained their ability to cleave the precursor viral protein, p55, into p41 and p24. This finding indicates that mutant PRs have altered host substrate specificity.

Sundararaj et al. show a mechanism where viral components can suppress innate immunity mediated by IFN-1, which is crucial for early control of a viral infection.[3] These findings give insight into how HIV-1 leverages its own reproductive strategy to suppresses immunity and how maintaining these basic functions as it co-evolves with antiviral medicines results in a choice between survival and becoming more vulnerable to immune clearance.[2]

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Beware the HIV Env, Which Comes to you in Glycosylated Coating

By Ambreen Niaman

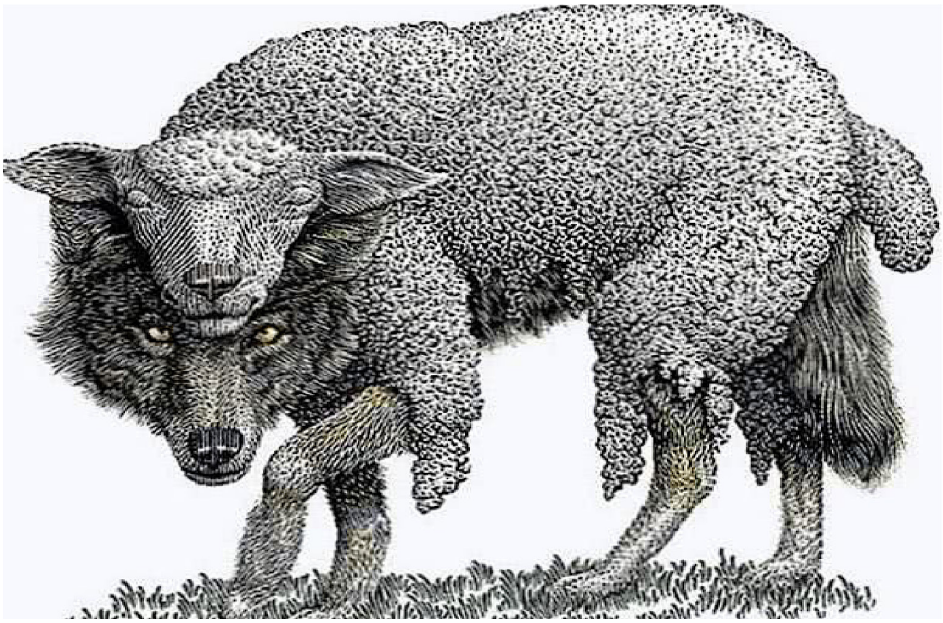
Microorganisms have evolved enormously over the years which contributed to their immune evasion strategies. Some examples from Human Cytomegalovirus (HCMV) include the inhibition of major histocompatibility complex (MHC-1) via a family of homologous genes encoded on the HCMV genomic unique short (US) region.[1] This is achieved by the prevention of intracellular transport of MHC-1 molecules by an endoplasmic reticulum (ER) glycoprotein US3, which is an immediate early (IE) gene within the US region of the virus. The US11 gene, which encodes an ER resident type-1 transmembrane protein, and is expressed in later phases of infection, downregulates the MHC-1 expression by dislocating newly synthesized class-1 molecules from the ER to the cytosol where N-glycanase and the proteosome cause degradation of this complex.[2]

However, in the universe of immune evasion, the Human Immunodeficiency Virus–1(HIV-1) is king. By targeting CD-4+ Helper T Cells, the virus scores a masterful blow by effectively crippling the keystone of the host's immune system, eradicating a central player in supporting both cell-mediated and humoral immunity. This is further compounded by the nearly unlimited diversity afforded to an envelope protein presenting an external surface that functions primarily as a shield covering the more sequence-restricted receptor-binding elements.[3]

This review examines work by Montefiori, Robinson, and Mitchell on how HIV-1 uses protein glycosylation to dress as a wolf in sheep's clothing.

The envelope (Env) glycosylation process by HIV-1 can be used to distinguish intra-clade and inter-clade variation and provides insight into how glycosylation affects viral immunogenicity. The Env genes of this virus encode two heavily glycosylated proteins located in the viral membrane. Following expression, a large precursor protein (gp160) is formed, which is then cleaved into an amino-terminal outer membrane gp120, and carboxy-terminal derived gp41.[4]

Montefiori et al. posit that the extensive glycosylation of HIV-1 Env proteins,



gp120/gp41, plays an important role in immune evasion by covering the neutralization epitopes and presenting only the glycosylated portion of these proteins to the host immune system.

The importance of protein glycosylation in HIV-1 Env proteins assuming its ovine appearance was evaluated by selectively interrupting N-linked glycoprotein processing and assessing the subsequent replication, infectivity, and pathogenicity of the virus.[4]

One way in which the virulence of HIV results comes from CD4-gp120 interactions which induces T cell- T cell fusion events to form syncytia. Specifically, syncytia are produced by the interaction of gp120 or gp41expressed on an infected cell with the CD4 expressed on neighboring cell surfaces. The involvement of CD4 and gp120 interactions was demonstrated using anti-CD4 antibodies and site-directed mutation of gp120 to inhibit syncytia formation in vitro.

Further investigation revealed that it was specifically the N-glycosylation of envelope proteins that was necessary for HIV-1 to express its Env proteins intact and also to exert its cytopathic effects. Coincubation of HIV-infected H9 cells with uninfected MT-2 cells, and the N-glycosylation inhibitors castanospermine (a potent inhibitor of some glucosidase enzymes), 1-deoxynojirimycin (an alpha-glucosidase inhibitor), 1-deoxymannojirimycin (a mannosidase-I inhibitor), or tunicamycin (an inhibitor of GlcNAc phosphotransferase which acts early in glycoprotein synthesis.) was assessed. After 24 hours, the reduction in syncytial formation and cytopathic effect was observed. In contrast, no syncytium formation was observed when untreated H9/HTLV-IIIB cells were mixed with MT-2 cells preincubated with inhibitors. This suggested that HIV-induced syncytium formation and its associated cytopathic effects are greatly dependent on glycoprotein processing.

These results support a role for N-glycosylation of HIV-I Env proteins as necessary for cytopathic effects. About 50% of Env mass is contributed by host-cell derived N-linked glycans which are considered a major protective shield against immune recognition.[5] Because glycans are, in general, less amenable to inducing humoral immune responses, these can mask conserved polypeptide epitopes making it difficult for antibodies to recognize viable epitopes.[6]

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Chikungunya Virus Refuses to be Complemented

By Guenaele Raphael

Immune evasion is a hallmark of a pathogens' virulence and pathogenicity. To be successful in a host, pathogens have developed different strategies to overcome the immune system. For example, human cytomegalovirus (HCMV) inhibits MHC class I antigen presentation which prevents cytotoxic T cells from recognizing viral and self-antigens.[1,2] Other viruses, such as Hepatitis C (HCV), suppresses the immune system by blocking important signaling pathways involving pattern recognition receptors such as Toll-like receptors or RIG-I like receptors,[3,4] while Bordetella Bronchiseptica can inhibit the MAP kinase pathway and Nf-kB activation.[5]

Chikungunya virus (CHIKV), a re-emerging mosquito-borne pathogen, also successfully evades and suppresses the host immune response.[6] This virus was first isolated and discovered in Tanzania in the early 1950s,[7] and since then has caused major outbreaks around the globe. The most recent outbreaks occurred in 2013 within the regions of North and South America which prompted a concerted effort to better understand the pathogenesis of this virus, particularly how CHIKV interacts with the immune system. Prior studies have shown that CHIKV can elicit massive secretion of IFNs as well as proinflammatory chemokines and cytokines,[8] which help in controlling progression and dissemination of the pathogen inside the host. Interestingly, CHIKV has developed various strategies to counteract the host's immune system, including the disruption of IFN signaling