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A Sophie's Choice between Viral Survival and Immune Evasion

By Cinthia Moncada

The immune system has one important role: to protect the body from outside invaders. It consists of intercommunicating subsystems of innate and adaptive cells and intercellular pathways that allow the body to fight off disease-causing organisms. One way it does so is by producing interferons, which elicit antiviral responses. Type-1 Interferons (IFN-1, IFN-α, and IFN-β) are part of the innate immune response released by cells in response to pathogen engagement. Specifically, TANK-binding kinase 1 (TBK1) is known to induce IFN-1 production as part of the innate immune response triggered through the pattern recognition receptor, retinoic acid-inducible gene I (RIG-I).[1] When TBK1 is activated, it homo-dimerizes and phosphorylates interferon regulatory factor 3 (IRF3), which translocates from the cytosol to the nucleus, where it serves as a transactivator of IFN-1 expression.[2]

Viruses have evolved a number of mechanisms to suppress the immune system in order to replicate within its host. One example of such a virus is the herpes simplex virus type 1 (HSV-1). Previous studies have shown that the HSV-1 protein, UL46, is able to downregulate TBK1- dependent antiviral innate immunity though a direct interaction.[3] This prevents TBK1 homodimerization and subsequent phosphorylation of IRF3, culminating in a failure to produce IFN-1. A second example of a virus that interferes with the signaling pathway that produces IFN-1 is Human immunodeficiency virus -1 (HIV-1). HIV-1 consists of several accessory proteins that can suppress the innate immune response including the HIV-1 protease (PR). PR is an enzyme that cleaves viral polyproteins into functional subunits and also interferes with host cell proteins to suppress IFN production. PR accomplishes this by cleaving TBK1, the key signaling component of IFN production pathways discussed above.[2] Sundararaj et al. demonstrated the PR proteolytic-mediated cleavage of TBK1 and how the resulting cleavage products were unable to activate IRF3 thus reducing IFN-1 production (Illustrated in **Figure 1**).

To determine if HIV-1 PR could interact and cleave other kinases, Sundararaj et al. screened a library of 412 human kinases using the wheat germ cell-free production system. To determine the interaction of PR and these kinases, a cleavage activity assay was conducted. This assay identified the cleavage of TBK1 by a two-color immunoblot. Cleavage of TBK1 was detected by a recombinant TBK1 with an N-terminal FLAG tag and a C-terminal biotin tag. When probed with reagents bearing red (anti-FLAG-alexa592 antibody)

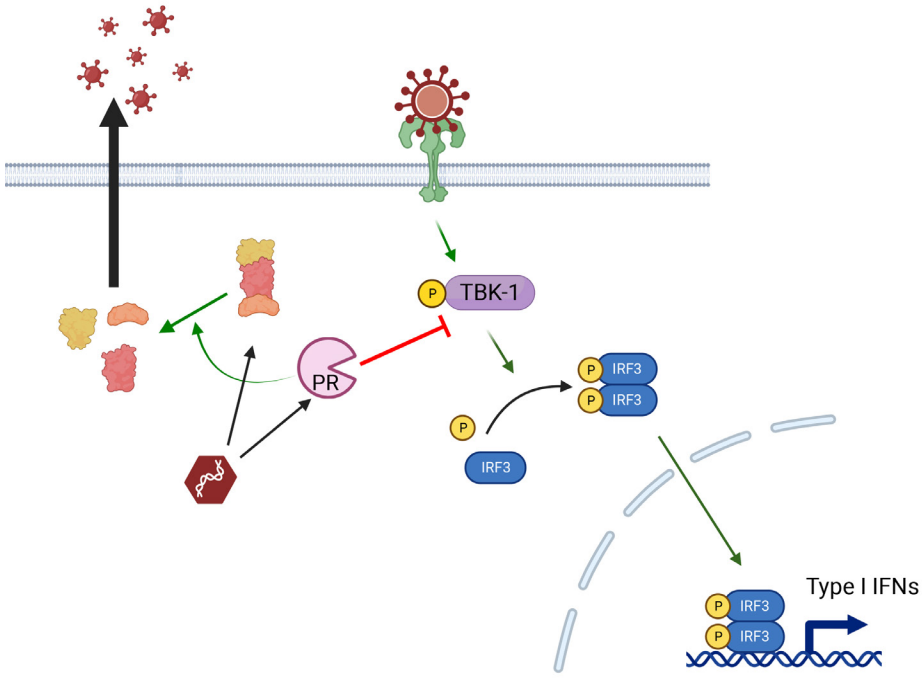

Figure 1 | Viral Protein Processing.
An HSV-1 viral genome synthesizes a protease to cleave its own polypeptide (left-side) critical to viral assembly and also interfere with interferon production mediated by TLR signaling (right-side).

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,