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]

References

1. Human cytomegalovirus inhibits antigen presentation by a sequential multistep process. https://www.pnas.org/doi/10.1073/pnas.93.20.10990doi:10.1073/pnas.93.20.10990.
2. Wiertz, E. J. et al. The human cytomegalovirus US11 gene product dislocates MHC class I heavy chains from the endoplasmic reticulum to the cytosol. Cell 84, 769–779 (1996).
3. Rademeyer, C. et al. Features of Recently Transmitted HIV-1 Clade C Viruses that Impact Antibody
4. Recognition: Implications for Active and Passive Immunization. PLoS Pathog 12, e1005742 (2016).
5. Montefiori, D. C., Robinson, W. E. & Mitchell, W. M. Role of protein N-glycosylation in pathogenesis of human immunodeficiency virus type 1. Proc. Natl. Acad. Sci. U.S.A. 85, 9248–9252 (1988).
6. Liang, Y. et al. Changes in Structure and Antigenicity of HIV-1 Env Trimers Resulting from Removal of a Conserved CD4 Binding Site-Proximal Glycan. J Virol 90, 9224–9236 (2016).

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

by the viral protease, nsP2, that can proteolytically cleave IFN.[9,10,11] Additionally, more recent research, from Nag et al., has shown an interaction between CHIKV and the human complement system.[12]

The complement system is made of a variety of plasma proteins that interact with one another to promote opsonization, neutralization, activation of phagocytosis, and other pro-inflammatory responses resulting in the assembly of the pore-forming, membrane attack complex (MAC). The complement system is activated through three distinct pathways, the classic pathway (CP), the lectin pathway (LP), and the alternative pathway (AP); each of which converge at the same effector molecule, C3 convertase. C3 convertase is an enzyme that can cleave a component of the complement system, C3 into C3a and C3b, which are a chemokine/mediator of inflammation and a potent opsonin, respectively.[12,13] This review article explores the work by Nag et al. that focuses on elucidating the mechanisms through which CHIKV can resist the human complement system by expression of a factor I-like activity.[12]

First, it was shown that CHIKV was able to activate the human complement system in a concentration-dependent manner. Briefly, a 2-fold serial dilution of sucrose-gradient-purified CHIKV was performed from 2.5 µg to 0.07 µg CHIKV and then incubated with normal human serum (NHS) for 45 min at 37ºC. Following the incubation, western blot analysis revealed that CHIKV was able to catalyze C3-to-C3a conversion between 0.15 µg and 0.31 µg CHIKV.[12] In a similar experiment,1.25 µg of CHIKV was incubated with NHS at different timepoints and showed time-dependent conversion of C3 to C3a from 5 minutes to 45 minutes. These results suggest that CHIKV is, in fact, able to activate the complement system in solution.

Since CHIKV demonstrated complement activation, the authors wanted to test if CHIKV is resistant to complement-mediated neutralization. To do so, complement-dependent neutralization assays were performed with the CHIKV. Briefly, CHIKV was incubated for 1h at 37ºC in different concentrations of NHS or heat-inactivated NHS (HI-NHS). The infectivity of these CHIKV samples were then determined by plaque assay on Vero cells. Compared to the virus-only control, incubations with high concentrations of NHS only reduced the number of plaques by 10 - 25%. To further substantiate this conclusion, a comparative analysis was performed with Chandipura virus (CHPV), a virus known for being sensitive to complement neutralization. As anticipated, CHPV exhibited a high sensitivity to complement-mediated-neutralization with a marked decrease in the number of plaques by up to 90%. These data further confirmed that CHIKV can resist complement-mediated-neutralization in vitro.

Recognizing the counterintuitive nature of CHIKV's ability to activate the complement system while also being relatively insensitive to complement-mediated neutralization, the authors sought to investigate if CHIKV was blocking complement-mediated neutralization by inhibiting deposition of complement components, specifically C3 and C4. Briefly, CHIKV samples, incubated with minimal essential media (MEM) or NHS at 37ºC for 1h, were ultracentrifuged and analyzed via western blotting to detect CHIKV proteins as well as C3 and C4. Both C3 and C4 were found to migrate with the viral fractions, suggesting that deposition of complement does in fact, occur. Analysis via electron microscopy further confirmed deposition of C3 and C4 components on CHIKV but in limited amounts which might be insufficient to trigger viral neutralization.

Since deposition of C3 and C4 on CHIKV does not trigger neutralization, the scientists decided to investigate the form of C3 that associates with the CHIKV virus. Normally, upon activation C3 is cleaved into C3a and C3b. However, this process can be abrogated by the presence of serine protease factor I, along with other cofactors such a factor H and CD46, forming the C3b inactivated form, iC3b; which occurs by the cleavage of the a-subunit of C3b. Interestingly, analysis via Western Blot reveals that the C3b component attached to the CHIKV virions lacks the a-subunit of C3b, suggesting the presence of a factor I-like activity by CHIKV. However, this factor I-like activity of CHIKV seemed to have no effect on C4b. Moreover, the degree of inactivation of C3b seems to be highly dependent on certain criteria: concentration of CHIKV, time of incubation, and the presence of the host cofactor H. Also, biochemical assays showed that cleavage of C3b is not due to the host factor I activity.

Overall, this paper suggests that CHIKV inhibits the activity of the complement system. These new findings offer valuable insights into the interaction of CHIKV and the complement system and contribute to its dissemination and progression in the bloodstream of infected hosts.

References

1. Ahn, K. et al. Human cytomegalovirus inhibits antigen presentation by a sequential multistep process. Proc. Natl. Acad. Sci. U. S. A. 93, 10990–10995 (1996).
2. Wiertz, E. J. H. J. et al. The Human Cytomegalovirus US11 Gene Product Dislocates MHC Class I Heavy Chains from the Endoplasmic Reticulum to the Cytosol. Cell 84, 769–779 (1996).
3. Li, K. et al. Immune evasion by hepatitis C virus NS3/4A protease-mediated cleavage of the Toll-like receptor 3 adaptor protein TRIF. Proc. Natl. Acad. Sci. U. S. A. 102, 2992–2997 (2005).
4. Meylan, E. et al. Cardif is an adaptor protein in the RIG-I antiviral pathway and is targeted by hepatitis C virus. Nature 437, 1167–1172 (2005).
5. Legarda, D., Klein-Patel, M. E., Yim, S., Yuk, M. H. & Diamond, G. Suppression of NF-?B-mediated ?-defensin gene expression in the mammalian airway by the Bordetella type III secretion system. Cell. Microbiol. 7, 489–497 (2005).
6. Caglioti, C. et al. Chikungunya virus infection: an overview. New Microbiol. 36, 211–227 (2013).
7. Ross, R. W. The Newala epidemic. III. The virus: isolation, pathogenic properties and relationship to the epidemic. J. Hyg. (Lond.) 54, 177–191 (1956).
8. Ng, L. F. P. et al. IL-1?, IL-6, and RANTES as Biomarkers of Chikungunya Severity. PLoS ONE 4, e4261 (2009).
9. Akhrymuk, I., Kulemzin, S. V. & Frolova, E. I. Evasion of the Innate Immune Response: the Old World Alphavirus nsP2 Protein Induces Rapid Degradation of Rpb1, a Catalytic Subunit of RNA Polymerase II. J. Virol. 86, 7180–7191 (2012).
10. Fros, J. J. et al. Chikungunya Virus Nonstructural Protein 2 Inhibits Type I/II Interferon-Stimulated JAK-STAT Signaling. J. Virol. 84, 10877–10887 (2010).
11. Bae, S., Lee, J. Y. & Myoung, J. Chikungunya Virus-Encoded nsP2, E2 and E1 Strongly Antagonize the Interferon-? Signaling Pathway. 29, 1852–1859 (2019).
12. Nag, J. et al. A Factor I-Like Activity Associated with Chikungunya Virus Contributes to Its Resistance to the Human Complement System. J. Virol. 94, e02062-19 (2020).