Chikungunya: *Silly Name, Serious Virus*

Photo Credit: National Institute of Allergy and Infectious Diseases's

TEXAS BIOMEDICAL RESEARCH INSTITUTE
HEALTH STARTS WITH SCIENCE

Transformation of Original Research Article

Guerrero-Arguero, I., Haj, T.R., Tass, E.S., Berges, B.K., Robison, R.A.(2020). A comparison of Chikungunya virus infection, progression, and cytokine profiles in human PMA-differentiated U937 and murine RAW264.7 monocyte derived macrophages. PLoS ONE 15(3). e0230328
https://doi.org/10.1371/journal.pone.0230328

Abstract

Chikungunya virus (CHIKV) is a mosquito-borne virus that causes rash, fever, and severe arthritis in humans. The arthritis can last for years post infection. Unlike humans, when murine models were infected with CHIKV, they exhibit an inflammatory response but only in tissues surrounding the inoculation site. Based on these different outcomes, it was suspected macrophages contributed to the systemic infection found in humans. In this study macrophages from murine and human cultures were infected with CHIKV. The replication rate and levels of RNA were analyzed and compared. The data show CHIKV replicates more effectively in human macrophages than in murine macrophages. Infected human macrophages were also shown to produce higher levels of CHIKV RNA leading to higher rates of systemic infection. Previous research has shown systemic infection increases inflammatory response in humans. The evidence from this study indicate CHIKV replicates more efficiently and induces higher levels of pro-inflammatory response in human macrophages when compared to murine macrophages. This suggests macrophages play a critical role in CHIKV inflammatory response in humans.

Background

The ***chikungunya virus*** (chick-oon-GOON-ya), abbreviated CHIKV is carried by mosquitoes. When a human is infected with CHIKV, symptoms can range from fever, rash, and in extreme cases can cause severe arthritis. Initially found in Southeast Asia and Africa, CHIKV cases have spread to South and Central America and Mexico. Mosquitoes make a microscopic puncture in the skin. As the mosquito feeds on blood, it injects an ***anticoagulant*** (an-tie-coh-AG-u-lant) to keep blood at the puncture site from clotting. The CHIKV enters the body with the anticoagulant.

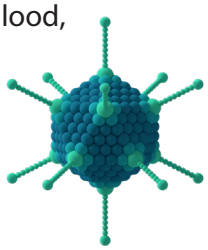

Image: Thomas Splettstoesser
Creative Commons

Chikungunya is an ***alphavirus***. Alphaviruses are a ***genus*** of RNA viruses that have a single strand of RNA inside its ***icosahedral capsid*** (eye-cosah-HEE-dral). The icosahedral capsid is a 20-sided structure whose surface is covered in triangular clusters of ***glycoproteins*** (gly-coh-PRO-teens). Cells most often infected with CHIKV are muscle cells, skin ***fibroblasts*** (cells that

help skin heal), and joint cells. In response to CHIKV infection, the immune system releases an antibody called ***immunoglobulin M*** (IgM). The IgM antibodies attach to the virus. When a mosquito transmits CHIKV to a human, not all people develop symptoms. Up to 28% of people infected with CHIKV will be asymptomatic. However, for those who develop symptoms, there are two phases: acute and chronic. The acute phase emerges at 7 days and lasts up to 12 days. The chronic phase has been reported to last up to 24 months. However, some people may develop conditions which last for years.

The amount of virus in blood plasma, known as the viral load, is measured by ***plaque forming units*** (pfu). With an acute infection, there are $1x10^6$ to $1x10^9$ pfu/ml of plasma. Greater numbers of pfu's in the blood plasma indicate a chronic phase of CHIKV infection. During both phases of CHIKV infection, IgM antibodies attack the CHIKV pfu's. However, during chronic CHIKV, there is evidence that the high levels of IgM antibodies needed to attack the pfu's over-stimulates the body's hormones. This may be responsible for the development of a devastating effect of CHIKV: rheumatoid arthritis.

The mechanism of how CHIKV infects cells is not completely understood. To gain insight into the mechanism of CHIKV infection, this study was designed to investigate and analyze how immune systems of different organisms respond to CHIKV infection.

The different immune responses of humans and mice prompted scientists to focus on macrophages. People who have CHIKV induced chronic arthritis have high quantities of macrophages in the infected joints. Prior to this study, it was known that macrophages are the first lines of defense to a mosquito bite in humans and mice. The question becomes what is the role of macrophages in CHIKV infection and systemic dissemination and why is this not observed in mice?

Methods

This study used ***murine*** (mice) macrophages to conduct an ***in vitro*** investigation to compare murine immune response to CHIKV infection to known human immune response to CHIKV. Mice are not naturally susceptible to CHIKV; however, there are strains of mice with a genetic mutation that suppresses their immune system. When immune-deficient mice were infected with CHIKV they exhibited different symptoms than humans.

Applying ***in vitro*** methods, scientists cultured murine and human macrophages. Macrophages from both organisms were infected with CHIKV. Two different rates of infection, referred to as ***Multiplicity of Infection*** (MOI) were tested. The MOI is a ratio of the level of attachment of infecting agents to macrophages.

Results

In this study, the scientists compared replication rates of CHIKV in human and murine macrophages to investigate the ***pathogenesis*** (path-oh-JEN-eh-sis) of the virus. Pathogenesis is the process by which a pathogen, like CHIKV, leads to the development of disease. In this study, a low MOI was compared to a high MOI in both human macrophages and murine macrophages. For the low and high MOI, cells were either "raw" murine macrophages or human (U937) macrophages (Fig. 1).

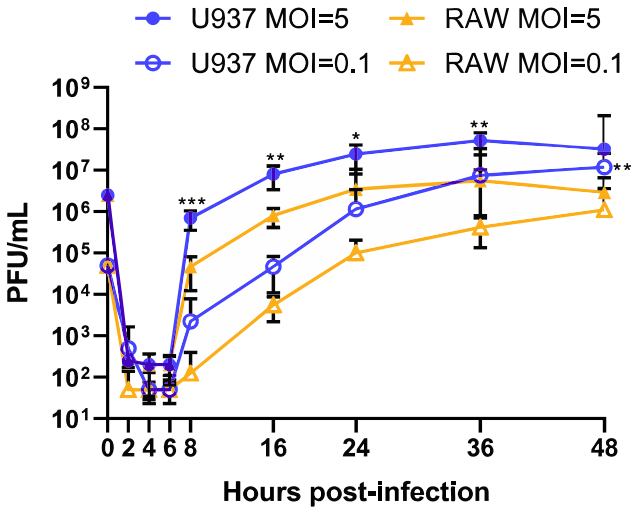

Figure 1: CHIKV replicates more efficiently in human macrophages (U937) than in murine macrophages. NOTE: 5 = high MOI; .01 = low MOI. N:.,.3ϑN:.,./ϑN:.,../ϑLQ* Imrqge Igdga_Ir,

Initial testing assessed the number of pfu's grown over time, indicating differences in replication. Next the scientists focused on the amount of viral RNA copies in macrophages over time. Keeping the low and high MOI's constant and measuring over the same number of hours post-infection, the scientists compared copies of viral RNA in murine and human macrophages (Fig. 2).

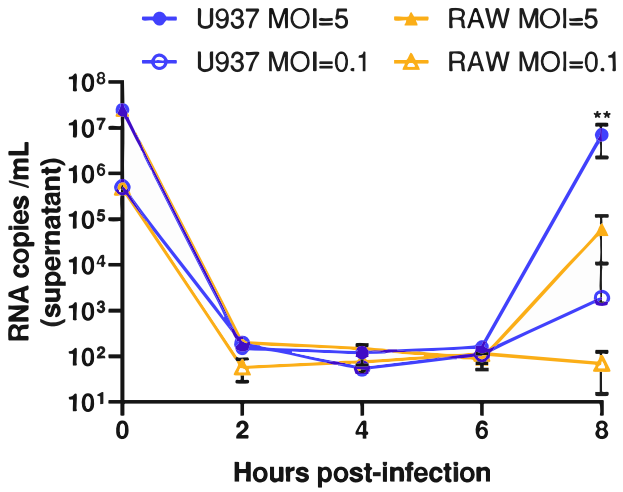

Figure 2: CHIKV replicates more efficiently in human macrophages than in murine macrophages. A) quantification of CHIKV RNA collected from 2 to 8 hours post infection (hpi). (P<0.05; **P<0.01;***P<0.001; NS, not significant.

Scientists observed a plateau of pfu's at 24 hours for both high MOI's for human and murine. To evaluate these results, scientists assessed the copies of viral RNA obtained over a 48-hour period post infection. Only the high MOI human macrophage reached and maintained a plateau at 24 hours (Fig. 3).

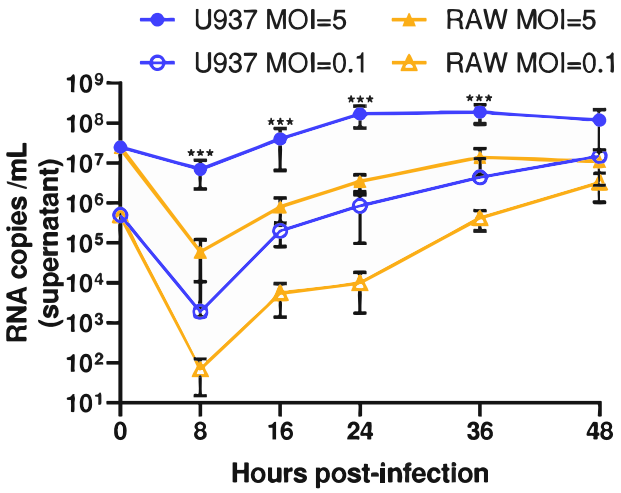

Figure 3: CHIKV replicates more efficiently in human macrophages than in murine macrophages. B) quantification of CHIKV RNA collected from 8 to 48 hours post infection (hpi). *P<0.05; **P<0.01;***P<0.001; NS, not significant.

Macrophages are one of the first lines of defense against infection. They secrete signals that either promote anti-inflammatory or pro-inflammatory responses. Inflammation is the key difference between human and murine response to CHIKV infection. Both human and murine immune systems have ***interleukins*** (IL). These are specialized molecules which act as messengers between immune cells, directing macrophage behavior and coordinating immune response to infections like CHIKV. In testing the inflammatory response, the role of IL's in human and murine macrophages were evaluated comparing infected macrophages to macrophages infected with "mock" viral infection. The human IL's tested caused an inflammatory response (Fig. 4A). Of the murine IL's, only two caused inflammatory response while all tested human IL's caused inflammatory responses (Fig. 4B).

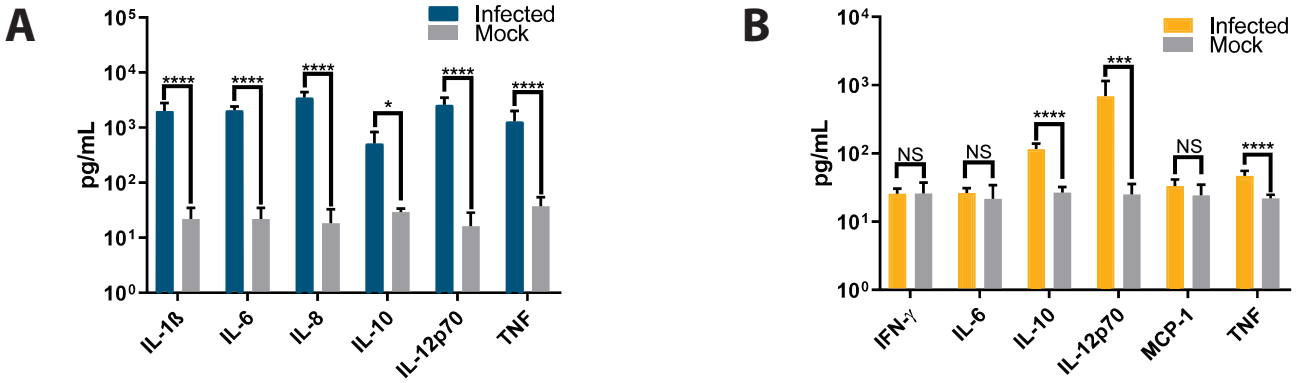

Figure 4: A) CHIKV infection induces pro-inflammatory response in human macrophages whereas B) only 2 murine IL's promote inflammatory response. NOTE: y-axis is measured in pico ml; tumor necrosis factor (TNF). *P<0.05; **P<0.01;***P<0.001; ****P<0.00001; NS, not significant.

Conclusion

Through this study, we investigated outcomes of CHIKV infection on macrophage lines from different species to identify differences to help provide evidence as to why murine responses to CHIKV differ from that of humans (Fig. 5).

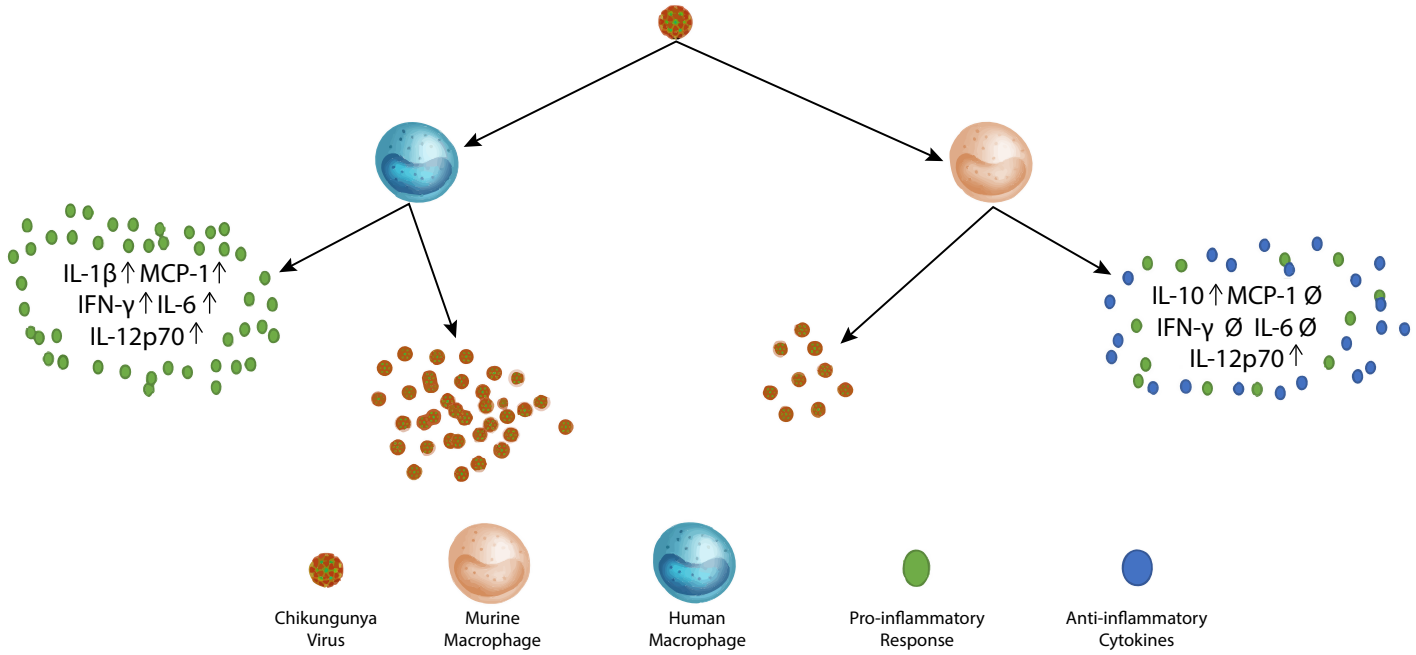

Figure 3: Graphic summary of CHIKV infection in human macrophages and murine macrophages. CHIKV infection in humans induces a higher pro-inflammatory response whereas CHIKV infection in murine models induces more anti-inflammatory responses.

The outcomes from this study provide evidence that CHIKV replicates easier in humans and may be attributed to a higher rate of inflammatory response promoted by the ILs. It has been suspected that CHIKV infection in humans induces a pro-inflammatory response triggering persistent joint pain and arthritis. The outcomes of this study provide supportive evidence for pro-inflammatory response to CHIKV.