Suppressors of Cytokine Signaling and Protein Inhibitors of Activated Signal Transducer and Activator of Transcriptions As Therapeutic Targets in Flavivirus Infections

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Flaviviruses cause significant human diseases putting more than 400 million people at risk annually worldwide. Because of migration and improved transportation, these viruses can be found on all continents (except Antarctica). Although a majority of the viruses are endemic in the tropics, a few [West Nile virus (WNV) and tick-borne encephalitis virus (TBEV)] have shown endemicity in Europe and North America. Currently, there are vaccines for the Yellow fever virus, Japanese encephalitis virus, and TBEV, but there is no effective vaccine and/or therapy against all other flaviviruses. Although there are intensive efforts to develop vaccines for Zika viruses, dengue viruses, and WNVs, there is the need for alternative or parallel antiviral therapeutic approaches. Suppressors of cytokine signaling (SOCS) and protein inhibitors of activated signal transducer and activator of transcription (STATs; PIAS), both regulatory proteins of the Janus kinase/STAT signaling pathway, have been explored as therapeutic targets in herpes simplex and vaccinia viruses, as well as in cancer therapy. In this review, we briefly describe the function of SOCS and PIAS and their therapeutic potential in flaviviral infections.

Color images are available online.

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Introduction

THE GENUS *FLAVIVIRUS* belongs to the viral family Flaviviridae and comprises about 70 arthropod-borne viruses, including the prototypical member, Yellow fever virus (YFV). The family got its name (“flavus”—meaning yellow in Latin) from the typical disease presentation of yellow coloration of the eyes and skin associated with yellow fever infections (Rogers and others 2006). Other members of the family include West Nile virus (WNV), dengue virus (DENV), Zika virus (ZIKV), and Japanese encephalitis virus (JEV) among many others and are transmitted by vectors—*Aedes* and *Culex* species of mosquito. Flaviviral infections are asymptomatic in a majority of affected persons but can cause severe human diseases. In severe cases, the infection usually leads to fever, myalgia, capillary leakage-hemorrhagic fever, encephalitis, and, in extreme cases, death, as there is no ant flaviviral therapy (Daep and others 2014).

Epidemiology of the Most Pathogenic Flaviviruses in Humans

YFV was first isolated in a Ghanaian patient by name Asibi, hence that strain was named after the patient (Gardner and Ryman 2010). About 170,000 cases of YFV occur in Africa annually with an estimated 60,000 associated deaths (Faria and others 2018). YFV infection is endemic in Africa Central and South America, but historically, there have been reported outbreaks in Europe and North America (Barnett 2007). YFV infection is thought to be maintained in the wild (sylvatic cycle) where nonhuman primates are infected through the bites of *Haemogogus* sp. mosquito, and humans living close to forest zones get infected when they are bitten by *Aedes* sp. mosquito (Faria and others 2018).

Although YFV has a very effective vaccine, there are still recorded outbreaks from time to time partly due to shortages and unavailability of the YF 17D vaccine. Only a few countries have integrated YFV vaccine into national vaccination programs and hence there is low vaccine coverage in at-risk populations (Barnett 2007). There is a peak in viremia about 2–3 days after YFV infection and disease presentation is varied ranging from asymptomatic to severe forms characterized by jaundice, hemorrhage, and kidney failure. To date, there is no specific antiviral therapy against YFV (Barnett 2007).

DENV is a global health threat and thought to be the most significant mosquito-borne viral disease (Murray and others 2013) infecting an estimated 400 million people annually (Bhatt and others 2013). DENV infection has been reported on almost all continents with more than 125 countries known to be DENV endemic (Murray and others 2013). DENV is transmitted by mosquito vectors *A. aegypti* and *A. albopictus*, which are known notoriously to feed during the daytime and also inhabit urban areas in endemic countries and hence keep DENV infection mostly in urban cities (Morrison and others 2008). Although DENV has been causing outbreaks many centuries ago, the virus was first isolated in 1943 in Japan (Messina and others 2014). There are 4 antigenically different serotypes of the virus, DENV 1, 2, 3, and 4, and infection is often misdiagnosed or confused with other febrile illnesses such as malaria in malaria-endemic

zones. DENV-infected people experience fever lasting for 2–7 days associated with myalgia, sore throat, and little rashes, which are mostly self-limiting and resolve in a couple of days (Murray and others 2013). Only about 1% of DENV-infected people will develop the severe form of the disease; dengue hemorrhagic fever which is often fatal (Dalrymple and others 2015). Survivors of DENV receive life-long immunity but to only that particular serotype that infected the individual and still puts one at risk of being infected with the other serotypes. There is the risk of antibody-dependent enhancement of dengue infection when one has previously been infected with another serotype of DENV than the serotype in a current infection (Flipse and others 2016).

Dengvaxia, a vaccine against DENV has been approved in about 19 countries and has been used in mass vaccination campaigns to more than 800,000 school children in the Philippines. Dengvaxia, however, has had some challenges after the manufacturer’s (Sanofi) analyses that the vaccine offers protection, especially to those who have had prior DENV infection, but puts others (those without prior DENV infection) at significantly increased risk of severe disease (Fatima and Syed 2018). There is no specific antiviral therapy against DENV (Bhatt and others 2013).

ZIKV was first isolated in a rhesus monkey in the Zika forest of Uganda, East Africa in 1947 (Wikan and Smith 2016). The primary route of ZIKV transmission is through bites of infected *A. aegypti* and *A. albopictus* mosquito but the transmission can be rather diverse; through the bite of an infected mosquito, maternal–fetal transmission, sexual intercourse, laboratory exposure, blood, blood product, and organ transplantation (Karkhah and others 2018). The first reported cases of ZIKV infection outside the African continent was in 2007 in the Yap state of Micronesia (Wikan and Smith 2016). Since then, there have been ZIKV outbreaks in Southeast Asia and the North, Central, and South Americas, with the most recent outbreak in Brazil recording over a million cases (Wikan and Smith 2016). ZIKV has, since the outbreak in Brazil, spread to the United States of America, United Kingdom, and some European countries and the majority of these are reported in travelers returning from South America (Zammarchi and others 2015).

ZIKV infection in pregnant women has been associated with congenital malformations, including microcephaly and Guillain-Barre syndrome, in babies born to such infected women (Cao-Lormeau and others 2016; Cauchemez and others 2016). As of 2015, it is thought that the ZIKV outbreak in the Americas and Pacific accounts for 500,000–1,500,000 suspected cases and a 20-fold increase in cases of microcephaly (Karkhah and others 2018). To date, 86 countries have reported mosquito-borne ZIKV infections; however, there is no specific treatment for ZIKV-infected people (Baz and Boivin 2019).

JEV is transmitted by the *Culex* spp. mosquito and outbreaks were reported in Japan in the late 1800s. JEV poses risk to about 3 billion people who live in the 24 most-affected countries. Annual reported cases are about 70,000 with about 10,000 associated fatalities (Mansfield and others 2017). Pigs have been reported to be nonsymptomatic viral reservoirs maintaining high viremia without impacting on animal health. JEV infection is often without symptoms,

but 1 in 250-infected people develop the severe disease (Yoshikawa and others 2016), characterized with neurological sequelae, including acute flaccid paralysis, mental confusion, seizures, and physical disabilities (Holbrook 2017). About 20%–30% of those with severe disease will result in fatalities and about a third of survivors will experience permanent intellectual and neurological problems (Yoshikawa and others 2016). JEV infection can be prevented by getting vaccinated with any of the 4 World Health Organization-approved vaccines, especially if one is travelling to JEV endemic areas in Asia. However, there is no antiviral therapy for JEV-infected persons and treatment is often by ensuring rest, hydration, and use of pain killers (Wang and others 2017). JEV infection seems to be a pediatric disease in unvaccinated populations, but in vaccinated populations, there are rare cases of infection in adults especially in the elderly (Campbell and others 2011; Connor and Bunn 2017).

Tick-borne encephalitis virus (TBEV) is prevalent in Europe and Asia and known to be transmitted by tick *Ixodes persulcatus* with the small animals that ticks feed on and humans as the reservoir and host (Randolph 2011). There are about 10,000–15,000 cases of TBEV infection reported annually in Europe and Asia (Dobler 2010). Higher cases of TBEV infection are reported in periods with increased tick activity—between April and November (Bogovic and Strle 2015). Transovarial and horizontal transmission of TBEV within the tick population that feed on the same rodent/small animal have been reported (Randolph 2011; Slovak and others 2014). Disease presentation is often sudden and characterized by a nonspecific febrile illness that later results in photophobia, encephalitis, and meningitis. About 30% of TBEV-infected victims will result in fatalities and survivors can have long-term problems, including poliomyelitis-like neurological sequelae (Holbrook 2017).

TBEV is more prevalent in adults than in children and the older the infected person is the more severe the outcome is (Bogovic and Strle 2015). The most effective way to prevent TBEV infection is by the use of vaccines. There are 2 efficacious vaccines available and approved for use in at-risk countries (Steffen 2016). However, there is no specific antiviral therapy against TBEV (Bogovic and Strle 2015).

WNV was first isolated in Uganda in 1937 (Smithburn and others 1940), but is now endemic in many parts of the world (Suthar and others 2013a). WNV is transmitted to humans through bites of *Culex* spp. mosquitoes that have fed on infected birds (Chancey and others 2015), however, a few WNV disease resulting from blood and/or organ transplantation have been reported (Hayes and others 2005). WNV is maintained in a natural cycle involving birds and mosquitoes. WNV was previously thought not to be pathogenic in birds, but there are reports of virulent WNV strains causing paralysis and death of birds in Israel (Root and Bosco-Lauth 2019). Although originated from Africa, it is now reported in countries on major migratory routes of birds with outbreaks occurring in Greece, Tunisia, Israel, Russia, and the United States of America (Root and Bosco-Lauth 2019). About 80% of WNV-infected individuals will not show any symptoms, and about 1% of infected people will develop severe neuroinvasive disease (Sejvar 2016). Reported cases in the United States of America indicate that all ages are at risk of WNV infection, but the rate of neuroinvasiveness seems to increase with age (Hayes and others 2005). There are currently no vaccines or antiviral therapy against WNV (Sejvar 2016).

Genome Organization, Viral Life Cycle, and Pathogenesis of Flaviviruses

Flaviviruses are enveloped positive sense, single-stranded RNA viruses, with a diameter of 40–50 nm (Crampon and others 2010) and a genome size of 9–12 kb. The genome encodes a single polyprotein that is cleaved into 3 structural and 7 nonstructural proteins by the host and viral proteases (Fernandez-Garcia and others 2009). The 3 structural proteins are—capsid (C), which is known to bind the viral genomic RNA; the membrane precursor (prM), which prevents premature viral fusion; and the envelope (E) protein, which interacts with cellular receptors to mediate virus entry and virion assembly (Diamond 2009). The nonstructural proteins are NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5 and are responsible for regulating viral replication, transcription, translation, and immune escape of host cell responses (Diamond 2009).

The virus enters permissive cells via receptor-mediated endocytosis and is channeled into endosomes. The exact host cell receptors that flaviviruses bind to are still yet to be completely elucidated, but it is known that flaviviruses budding out of host cells retain molecules of phosphatidylserine and phosphatidylethanolamine, which are components of host cell inner membrane. Phosphatidylserine receptors such as T cell immunoglobulin and TYRO3, AXL, and MERTK are thought to play key roles during flaviviral invasion into host cells (Oliveira and Peron 2019). Acidic pH in the endosomes causes a trimerization of the viral E protein that enables a fusion of the viral and host membranes (Mukhopadhyay and others 2005). Upon fusion, the capsid and viral RNA dissociate, and the released viral RNA utilizes the host endoplasmic reticulum (ER) membrane as a bed for viral transcription.

The viral genome encodes a polyprotein that is co- and posttranslationally processed into the various structural and nonstructural proteins by viral proteases (NS2B/NS3) and host cell signalases (Fernandez-Garcia and others 2009). After translation of the viral proteins, the NS5 protein, which acts as an RNA-dependent RNA polymerase, uses the viral genomic RNA to synthesize a minus-sense strand RNA that is used as a template for making viral positive-sense strand RNA to be incorporated into new viruses (Brinton 2002). Viral assembly takes place on the ER membrane and the noninfectious immature virions are transported through the Golgi apparatus and acted on by host proteases to form mature infective viruses. Mature viruses exit infected cells via exocytosis to infect other neighboring cells (Mukhopadhyay and others 2005).

Innate Immune Response to Flaviviral Infections

Considering that the majority of flaviviral infected individuals remain asymptomatic suggests that the innate immune system plays an essential role in curtailing viral replication and disease pathogenesis. Type-I interferons (IFNs, a family of cytokines) are one of the most prominent and successful components of the innate immune system in the fight against viruses. When flaviviruses invade a cell, the ssRNA genome and/or intermediary dsRNA is recognized as pathogen-associated molecular patterns by host cell pattern recognition receptors (PRRs) such as toll-like receptors (TLRs) and retinoic-acid inducible gene 1 (RIG-I)-like receptors (RLRs) to trigger a cascade of signaling pathways, leading to the production of type-I IFNs (IFN- α/β) (Wu and Chen 2014).

Flaviviral Sensing Mechanisms

To fight off invading viruses, the cell must be able to “sense” or recognize nonself particles or molecules to trigger an immune response. Cells can recognize nonself RNA and/or DNA via pathogen recognition receptors that are present in the cytoplasm and endosomes. Flaviviral replication cycle transit through the endosomes and occurs entirely in the cytoplasm, although recent studies show that some viral proteins such as NS5 can be found in the nucleus (Lopez-Denman and Mackenzie 2017). The most widely studied of these PRRs is the TLR. Currently, 10 functional human TLRs have been discovered and can be grouped into 2, based on their localization—TLRs 1/2/4/5/6/10 are localized on cell membranes and TLRs 3/7/8/9 are in endosomes.

TLRs can also be grouped into 2, based on the adaptor protein signaling pathway, they activate. Excerpt for TLR3, when TLRs sense pathogen structural motifs, they form homodimers or heterodimers (TLR1/2 or TLR2/6) and then recruit the adaptor protein myeloid differentiation primary response protein 88 (MyD88) via their Toll IL-1 receptor domain. MyD88 then interacts with interleukin (IL)-1/4-receptor activating kinase (IRAK1/4) and then IRAK 4 phosphorylates and activates IRAK2. IRAK4/2 dimerises and leaves the TLR/MyD88 complex to associate with tumor necrosis factor receptor-associated protein 6 and eventually leads to effective activation of nuclear factor kappa-light-chain enhancer of activated B cells (NF- κ B) and mitogen activated protein kinase. The potent transcription factor NF- κ B leads to the production of copious amounts of type I IFNs and proinflammatory cytokines and chemokines (Arleevskaya and others 2019). TLR3 is MyD88-independent and signals through TRIF (Toll/IL1-R domain-containing adaptor inducing IFN- β) pathway to activate the transcription factors IFN regulatory factors 3 and 7 (IRF3 and 7). IRF3/7 is translocated into the nucleus to bind their response elements leading to the production of IFN- β and proinflammatory cytokines (Ullah and others 2016). TLR4 can activate both MyD88 and TRIF signaling pathways (Ullah and others 2016).

The role of TLR sensing of flaviviruses cannot be overemphasized as knock-out mice lacking specific TLRs, for example, TLR3 (Chugh and others 2014) and TLR7 (Sabouri and others 2014) are significantly prone to severe pathogenesis by WNV infection. DENV activated and upregulated the expression of TLR2 and 6 in human peripheral blood mononuclear cells (PBMCs) and resulted in higher levels of IL6 and tumor necrosis factor (TNF)- α (Chen and others 2015). In a confirmatory experiment, blocking TLR2 and TLR6 with antibodies reduced the expression of IL6 and TNF- α in lipopolysaccharide-induced PBMCs (Chen and others 2015). However, TLR signaling can be more complex than it seems as the overstimulation of TLRs and can have rather pathogenic sequelae rather than eliciting protection. Activation of TLR3 has been associated with an increased inflammatory response that causes a breakdown in the blood–brain barrier to facilitate entry of WNV into the central nervous system (CNS) to cause severe encephalitis (Wang and others 2004). Also, TLR6 knock-out mice have been reported to have increased survival ability to DENV infection (Chen and others 2015).

Other PRRs involved in sensing flaviviruses include the RLRs; RIG-I and melanoma differentiation-associated gene 5 (MDA5) are capable of recognizing flaviviral dsRNA. Once RLRs recognize nonself (viral) RNA, they are acti-

vated and translocated into the mitochondria where they interact with the mitochondrial antiviral signaling (MAVS) protein. The RIG-I/MAVS or MDA5/MAVS complex then recruits other adaptor proteins and kinases to form the IFN- β enhanceosome and eventually lead to the production of proinflammatory cytokines and IFN- β and consequently, the synthesis of many IFN-stimulated genes (ISGs) as a downstream effect of IFN-Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signaling that restricts viral replication (Quicke and others 2017). RIG-I and MDA5 sensing of flaviviruses are critical in the immune response, as knockdown of RIG-I and MDA5 in Huh7 cells result in increased DENV replication (Nasirudeen and others 2011). However, DENV has been reported to evade RIG/MAVS- and MDA5/MAVS-directed innate response by blocking IRF3 phosphorylation (Dalrymple and others 2015). RIG-I knock-out has been associated with delayed early immune response to WNV infection signifying that the RIG-I signaling pathway is required in the innate immune response to WNV infection (Fredericksen and Gale 2006).

NOD-like receptors (NLRs), another PRR, have also been reported to be activated in WNV and DENV infections and lead to the formation of the inflammasome complex and subsequent production of inflammatory markers belonging to the IL-1b family (Suthar and others 2013b). Similar results of NLR (inflammasome) activation have also been reported in ZIKV infections (Khaiboullina and others 2017).

In a rather uncharacteristic event, DENV has been reported to trigger the cyclic GMP-AMP synthase/stimulator of IFN genes (cGAS/STING) signaling pathway, which is typically known to sense DNA viruses. It has been reported that during DENV infection, mitochondrial DNA is spilled into the cytoplasm and this activates the cGAS/STING pathway to stimulate the production of type-I IFNs (Schoggins and others 2014). Moreover, DENV nonstructural protein NS2B has been reported to specifically degrade cGAS in infected cells as a means of evading host innate IFN-response (Schoggins and others 2014). However, whether this non-canonical approach observed with DENV is reflected in other flaviviruses is yet to be reported.

Flaviviral ss/dsRNA sensing by TLRs, RLRs, and other PRRs results in the secretion of type I IFNs. Secreted IFNs interact with IFN receptors to trigger the JAK/STAT pathway leading to the production of over a thousand ISGs with antiviral properties (Fernandez-Garcia and others 2016).

IFN-JAK/STAT signaling

Type-I IFNs, IFN- α/β , bind to their receptors IFNAR1 and IFNAR2, causing autophosphorylation and activation of JAKs (Tyk2 and JAK1) (Ivashkiv and Donlin 2014). Activated JAKs phosphorylate IFNAR receptors to create a binding pocket to recruit JAK-phosphorylated STAT2 (Levy and Darnell 2002). Activated STAT2 then recruits STAT1 to form a STAT1/STAT2 heterodimer, which interacts with a third molecule, IRF9, to form the transcription complex IFN-stimulated gene factor 3 (Best 2017). The complex is translocated into the nucleus where it interacts with transcriptional coactivators such as the cAMP-response element-binding protein (CREB)-binding protein (CBP/p300) (Zhang and others 2008) at the IFN-stimulated response elements to cause upregulation of the transcription of over a thousand

antiviral molecules such as interferon-induced protein with tetratricopeptide repeats 1 (IFIT1) and interferon-induced transmembrane protein (Aaronson and Horvath 2002; MacMicking 2012; Best 2017).

Some of the important regulators of the type I-IFN-signaling pathway are suppressors of cytokine signaling (SOCS) family of proteins and protein inhibitors of activated STATs (PIAS), both of which can affect a negative feedback regulation on the IFN-signaling pathway. Physiologically, host cells express SOCS and PIAS to limit the exaggeration of inflammatory signals by channeling signaling proteins through proteasomal degradation while still maintaining a good-enough immune response (Yoshimura and others 2007). Viruses have found ways to exploit the functions of SOCS proteins (Fig. 1) by robustly causing the overexpression of the proteins, which then limits the host immune response and facilitates viral replication, assembly and eventual severe disease pathogenesis (Akhtar and Benveniste 2011).

Insects utilize the JAK/STAT pathway as 1 of 3 innate immune mechanisms against invading pathogens. Orthologs to human JAKs and STATs have been identified in mosquitoes and *Drosophila melanogaster* and have been shown to play key roles in antiviral immunity against the *Drosophila* C virus and DENV in *A. aegypti* mosquitoes (Conn and others 2016). Mammalian SOCS and PIAS, which are negative regulators of JAK/STAT, have been identified in *D. melanogaster* (Arbouzova and Zeidler 2006) and *A. aegypti* (Souza-Neto and others 2009) to play similar functions.

The JAK/STAT-IFN signaling pathway is highly regulated and some flavivirus-induced negative regulators are summarized in Fig. 1.

Noncanonical signaling pathway

The JAK proteins (JAK1, JAK2, JAK3, and Tyk2) are activated by phosphorylating each other at specific tyrosine residue (Y701) to perform their canonical JAK/STAT signaling through type I and IFN-I pathways. However, recent evidence from studies performed in mice that lack this phosphorylation activation site points to other noncanonical functions of cytokine signaling that does not require phosphorylation of JAK proteins (Majoros and others 2017). Mice lacking STAT1 or humans with polymorphisms in STAT1 still have some residual type I IFN and IFN-g signaling activity, and this supports the noncanonical IFN signaling phenomenon (Gil and others 2001). Unphosphorylated STAT3 was the first STAT protein to be identified to function without necessarily being activated by phosphorylation because it was observed to be localized in the cytoplasm and nucleus (without being phosphorylated). The noncanonical IFN signaling pathway has been reviewed by Majoros and others (2017) and Li (2008).

Structure and General Function of SOCS

Cytokine signaling via the JAK/STAT pathway induces the expression of the SOCS proteins and inhibits the same pathway in a negative feedback loop. Eight proteins pertain to the SOCS family; cytokine-inducible SH2-containing protein (CIS) (Hunter and others 2016), and SOCS1–7 (Linossi and others 2013). Each of the 8 proteins contains a central SH2 domain, a C-terminal SOCS box, and an N-terminal with different lengths and divergent sequences

FIG. 1. Flavivirus-induced SOCS protein antagonism of types I and III interferon signaling through the Janus kinase/STAT pathway. Color images are available online.

(Akhtar and Benveniste 2011) (Fig. 2A). The SOCS-box is a 40-amino acid motif that serves as a docking site for adaptor proteins such as Elongins B and C and the scaffold protein Cullin5. SOCS 1 and 3 have a unique 12-amino acid residue at the N-terminal called the kinase inhibitory region (KIR), which inhibits JAK tyrosine kinase activity by covering the substrate-binding pocket on JAK (Ying and others 2019).

Much of our current understanding of the SOCS protein family is based on the well-researched CIS and SOCS 1,2, and 3, with very little known about the true functions of SOCS 4,5,6, and 7 (Linossi and others 2013).

The SH2 domains of SOCS bind to specific phosphorylated tyrosine residues in their target proteins, followed by the SOCS box interacting with a complex of proteins comprising of RING-box-2 (RBX2), Culin5, and Elongins B and C to form an E3 ubiquitin ligase. The resulting complex facilitates the ubiquitination of the SOCS-bound target protein by channeling the target protein through the proteasomal degradation pathway (Akhtar and Benveniste 2011).

SOCS1 SH2 domain is a potent inhibitor of JAKs activity by colocalizing with microtubule organizing complex (MTOC) and channeling JAKs through proteasomal degradation using the MTOC-associated 20S proteasome (Vuong and others 2004). SOCS1 and SOCS3 compete with STAT1 phosphorylation by interacting with JAK through their KIR to inhibit JAKs tyrosine kinase activity (Yoshimura and others 2007). CIS and SOCS2 have also been found to compete with and sterically interrupt the binding of recruited STAT proteins to the cytoplasmic domain of IFN receptors (Mansfield and others 2010; Akhtar and Benveniste 2011).

Apart from the JAK/STAT pathway, some SOCS proteins have been reported to regulate other signal transduction pathways. SOCS1 acts as a ubiquitin ligase to destabilize p65 subunit of NF- κ B by ubiquitin-mediated proteolysis and

thereby interrupting the signaling function of NF- κ B (Ryo and others 2003). SOCS1 also interferes with NF- κ B signaling by binding to and degrading myelin and lymphocytes protein (Mansell and others 2006) and IRAK (Dimitriou and others 2008), both of which are essential adaptor proteins involved in NF- κ B signaling. These events are summarized in Fig. 1.

Structure and Function of PIAS

PIAS is a family of 4 proteins; PIAS1, PIASx (or PIAS2), PIAS3, and PIASy (or PIAS4). The proteins were initially discovered to inhibit STATs, but they are also able to positively and negatively modulate a myriad of other proteins (about 60) involved in many cellular processes such as transcription repression, cell cycle control, DNA repair, and NF- κ B regulation (Murphy and others 2010; Conn and others 2016). PIAS proteins have an N-terminal scaffold attachment factor A and B (SAP) domain, PINIT motif, RING domain, an acidic domain, a SUMO-interacting motif (SIM), and a variable serine/threonine domain (Fig. 2B). The PINIT motif, SAP, and RING domains all contribute to the nuclear localization of PIAS. The SAP domain is made up of 4 alpha-helices that bind DNA at A/T-rich regions (Okubo and others 2004). The SAP domain also contains an LXXLL motif that binds to nuclear receptors and transcription factors (Shuai 2006). PIAS modulate the JAK/STAT signaling pathway via Small Ubiquitin-like modifier (Getts and others)-dependent or SUMO-independent mechanisms. They are known to be involved in sumoylation (Kotaja and others 2002) (attaching SUMO) of lysine residues in interacting proteins such as STATs to modify their function, change their cellular location/trafficking or preventing them from interacting with DNA.

FIG. 2. Schematic structure of SOCS (A) and protein inhibitors of activated STATs (B) proteins. Gray indicates no specialized protein motif. SOCS, suppressors of cytokine signaling; STAT, signal transducer and activator of transcription. Color images are available online.

PIAS1 has been reported to inhibit DNA-binding activity of pSTAT1 to repress STAT1-mediated gene expression in a dose-dependent and specific manner, as neither PIAS3 nor PIAS2 was able to repress STAT1-mediated gene expression (Liu and others 1998). PIAS1^{-/-} null mice model exhibited an enhanced immune response to mouse herpes viral infection and a 90% reduction in viral titers 72 h post-mouse herpesvirus infection in Pias^{-/-} mouse macrophages (Liu and others 1998). PIAS1 binds to p65 subunit in the nucleus to inhibit the expression of NF- κ B modulated genes, whereas these genes are significantly enhanced in Pias^{-/-} bone marrow-derived macrophages (Liu and others 2005).

PIAS1 and PIAS3 have been shown to prevent DNA-binding by STAT1 (Liu and others 1998) and STAT3 (Chung and others 1997), respectively, to inhibit STAT1- and STAT3-mediated transcription in a SUMO-independent manner. Although the actual mechanism of repression is not entirely clear, there are 3 possible propositions; (i) PIAS1 could bind to DNA and prevent STAT1 from binding to the DNA-binding site (Liu and others 1998), (ii) PIAS1 can directly bind and change the conformation of STAT1 rendering it incapable to bind the DNA or (iii) PIAS1 can cause monomerization of STAT dimers (Liu and others 1998; Murphy and others 2010). Another SUMO-independent mechanism is the phosphorylation of PIAS1 at Ser90 residue to repress STAT1 and NF- κ B transcriptional activity by binding to STAT1 and NF- κ B promoter sites and eventually reducing the levels of ISGs (Liu and others 2004, 2007).

PIAS2 has been shown to interact with phosphorylated STAT4 in IL-12-stimulated human Kit225 cells, which express IL-12 receptors, thereby inhibiting STAT4-mediated gene transcription without blocking the DNA-binding activity of STAT4 (Arora and others 2003).

In IFN-treated 293T and U2OS cell lines, PIAS4 has been shown to repress STAT1-mediated gene expression by binding to STAT1, but without inhibiting the DNA-binding activity of STAT1 (Liu and others 2001), in contrast to what has been observed in PIAS1 inhibition of STAT1. Another mechanism of PIAS repression of transcription factors is the sequestration of transcription factors in subnuclear localizations; for example, PIAS4 represses the transcription activity of lymphoid enhancer binding factor 1 (LEF1) in a SUMO-dependent manner by sequestering LEF1 to subnuclear structures called nuclear bodies (Sachdev and others 2001).

Viral Exploitation of SOCS and PIAS Functions

The JAK/STAT pathway is a critical piece in innate and adaptive immune responses and SOCS and PIAS being regulators of this critical step present a bottleneck that could be exploited in the pathogenesis of diseases that have immune malfunction as basic etiology such as cancers and viral infections. The initial physiological response to viral infections is the rapid nonspecific innate immune response characterized by the complement system (not discussed here) and the production of cytokines that signal through the JAK/STAT pathway to generate an elaborate set of many antiviral molecules to counter viral replication. This is then followed by the slow and more antigen-specific adaptive immune response. The adaptive response is the cell-mediated immune response where cytotoxic T cells (CD8+) recognize viral antigens presented on major

histocompatibility complex class I receptors on infected cells and target them for destruction. Also, in the adaptive immune response is the humoral immune response where B cells with the help of CD4+ T cells recognize mostly cell-free viruses and produce antibodies that recognize specific viral epitopes, aggregate viruses, and halts further cell infections (Dolasia and others 2018).

Viruses have evolved many strategies to evade this complex immune system. In this section, we will discuss viral-induced upregulation of SOCS and PIAS as an immune evasion strategy utilized by viruses and how their immune regulatory functions in both innate and adaptive response have been exploited to promote viral replication.

Suppressors of cytokine signaling

More than 80% of hepatitis C virus (HCV) infected individuals develop chronic infection suggesting that HCV can evade the immune response to establish chronicity. Historically, Bode and others (2003) were first to describe SOCS3 upregulation as an evasion mechanism used by HCV, where they showed that HCV core protein can upregulate SOCS3 expression in hepatocytes (HepG2) to inhibit nuclear translocation of activated STAT1. The main treatment regimen of HCV is the administration of IFN- α and ribavirin (antiviral), but about half of those treated do not respond to treatment and this has been attributed to viral and/or host factors (Cavalcante and Lyra 2015). HCV genotype 1 has been associated with nonresponse to treatment, and by far, the most significant host factor associated with nonresponse is the expression level of SOCS3 (Walsh and others 2006; Kim and others 2009). However, other studies have reported elevated levels of SOCS1 and not SOCS3 in chronic HCV-infected people (Imanaka and others 2005), but what remains clear is that expression levels of SOCS proteins hamper HCV treatment and levels of SOCS proteins could be predictive markers for treatment success (Aslam and others 2016).

Tat protein of HIV-1 (a retrovirus) can upregulate the expression levels of SOCS3 to inhibit IFN signaling by reducing the phosphorylation of STAT1 and STAT2 as well as the expression of antiviral molecules PKR and ISG20 with a consequent increase in viral replication *in vitro* in macrophages (Akhtar and others 2010). There is widespread use of highly active antiretrovirals in HIV-1 infected people, however, about half of the infected people will develop cognitive and neurological problems generally termed as HIV-1-associated neurocognitive disorder that may eventually deteriorate to HIV-associated dementia in some (Gonzalez-Scarano and Martin-Garcia 2005). Although HIV does not infect neurons, microglia cells (resident macrophages in the brain) are infected and released inflammatory signals can affect neuronal cells of the brain (Gonzalez-Scarano and Martin-Garcia 2005). HIV-1 replication in microglia cells in HAD macaque model is kept in check by the IFN- β innate signaling, but there is a sudden rebound of viral replication affecting the CNS in this macaque model. This rebound correlated and has been associated with increased expression of SOCS3 that reduces innate immunity and then enhances viral replication as has been observed with HIV-1 Tat protein in *in vitro* studies mentioned earlier (Akhtar and others 2010). Increased bone loss (osteoporosis) lead to bone fracture in HIV-1-infected people and this

has been associated with increased osteoclasts (cells responsible for bone resorption) differentiation. It has been demonstrated that HIV-1 drives increased expression of SOCS1 to interfere with the inhibitory effect of IFN- γ on osteoclast precursor cells in an NF- κ B mechanism that drives the accelerated differentiation into osteoclasts leading to significant osteoporosis in HIV-1-infected people. The authors postulate that modulating expression of SOCS1 could be a possible treatment option to slow down bone loss in HIV-1-infected people (Lafferty and others 2014). HIV-1 like other viruses are obligate parasites and hence depend heavily on host molecular systems to make virus progeny. It has been reported that HIV-1-induced SOCS1 bind to Gag polyprotein of the virus to stabilize it and enhance the cellular trafficking of this protein within cells to viral replicating centers leading to higher HIV-1 titers. (Ryo and others 2008) The therapeutic potential of this observation is discussed in later sections.

Herpes simplex virus 1 (HSV-1) infects more than half of the world's population, but it is mostly asymptomatic because it is kept in check by the innate and adaptive systems through IFN- γ produced by dendritic cells (DC) and CD8+ T cells, respectively. HSV-1, however, has evolved many immune evasion mechanisms to come out of latency into active replication to cause topical lesions, which put infected people at risk of contracting other viral infections such as HIV. Many studies have demonstrated that one of the immune evasion mechanisms used by HSV-1 is to cause an increase in the expression of SOCS1 and SOCS3 (Yokota and others 2004; Frey and others 2009). SOCS1 mRNA and protein were significantly elevated with reduced phosphorylation of STAT1 in HEL30 keratinocytes infected with HSV-1, and these cells were refractory to establishing antiviral state after IFN- γ treatment, whereas fibroblasts cells L929 infected with HSV-1 had low levels of SOCS1 and responded to IFN- γ therapy suggesting differential expression of SOCS1 in different cell types (Frey and others 2009).

A SOCS1 peptide antagonist and how the authors applied this as a possible therapy to reduce HSV-1 replication are discussed in a later section. The severe form of HSV-1 infection is encephalitis. When SOCS2^{-/-} mice were infected with HSV-1, more of such mice survived compared to the mice with intact SOCS2, which had significantly elevated expression of SOCS2 upon HSV-1 infection. This suggests that HSV-1 induces SOCS2 as an evasion strategy in HSV-1 encephalitis (da Cunha Sousa and others 2016).

SOCS3 expression is also elevated in liver tissues of Hepatitis B virus (HBV)-infected patients studied with immunohistochemistry. Surprisingly, STAT3 which is interfered by SOCS3 was not downregulated with elevated SOCS3, but was rather constitutively activated in Huh cells infected with HBV (Koeberlein and others 2010). Again, the authors observed no increase in SOCS1 levels of Huh-infected cells, but activated STAT1 was reduced. They then posit that the dysregulated JAK/STAT/SOCS signaling could account for HBV pathogenesis and malignant liver transformations (Koeberlein and others 2010). HBV-induced upregulation of SOCS1 and SOCS3 have also been implicated in resistance to HBV therapy (Du and others 2014). Constitutive activation of JAK/STAT signaling promotes cell proliferation and creates a milieu for tumor development. Also, colleagues who studied methylation

status of *SOCS1-7* and *CIS* genes in 116 HBV-related hepatocellular carcinoma (HCC) observed that *SOCS2* gene was about 56% hypermethylated in tumor tissues and 54% hypermethylated in adjacent nontumor tissues. This suggests that *SOCS1* gene loss is implicated in HBV-induced HCC (Zhang and others 2014; Fu and others 2016).

In U937-derived macrophage cell line, DENV induced expression of SOCS1 and 3 by about 400 folds than levels in uninfected control cells. Increased expression of SOCS 1 and 3 occurred concurrently with a significant increase in viral infectivity of U937-derived macrophages. A concomitant reduction in levels of phosphorylated STAT2 (pSTAT2) and reduced nuclear translocation of phosphorylated STAT1 (pSTAT1) was also observed and together further inhibited antiviral immune response (Estrada-Jimenez and others 2016). An alternative route of inducing ISGs via type-III IFN (IFN- λ) is inhibited by the expression of SOCS1 and SOCS3 upon DENV infection of C33-A epithelial cells (Palma-Ocampo and others 2015).

JEV, one of the flaviviruses that causes neurological disease, has been demonstrated to exploit SOCS proteins for continuous viral replication *in vitro* and *in vivo*. JEV survivors still experience viral persistence many years after recovery, suggesting that the virus has evolved evasion strategies to overcome the immune system (Kundu and others 2013). JEV induced higher expression of SOCS3 in RAW264.7 cells and the brain of suckling mice injected with JEV subcutaneously could contribute to JEV-induced brain injury as shown by their histological and immunohistochemistry assays (Li and others 2014). Kundu and others (2013) observed a steady elevation in levels of SOCS1 and SOCS3 in macrophages obtained from the spleen and peritoneum of JEV-infected mice models. JEV-infected murine primary macrophages released cytokines (IFN- α) that activated adaptive immune response in about 6 h postinfection, but the response is transient as there was an exponential rise in expressed levels of SOCS1 and SOCS3, which acts as negative regulators of cytokine and chemokine production; drastically reducing levels of secreted cytokines such as IFN- α to basal levels within 24 h (Kundu and others 2013).

WNV and TBEV are other neurotropic flaviviruses that can show persistence in infected humans many years after symptoms have elapsed. They can be latent in kidneys as one study has suggested after detecting WNV in the urine of infected Americans a year after symptoms (Murray and others 2010). In mice models separately infected with WNV or TBEV, there was significant upregulation of SOCS1 (28-fold and 17-fold for WNV and TBEV, respectively) and SOCS3 (33-fold and 28-fold for WNV and TBEV, respectively) in the brain of infected mice, which suppressed the effect of cytokine response, possibly as a neuroprotective measure, but this "lowered the barrier" for encephalitis-causing viruses (WNV and TBEV) to spread into the brain to cause disease. It has been suggested that IFN- γ signaling in the CNS can cause some immunopathological defects (Getts and others 2007) and increases in expression of SOCS proteins will be expected to counteract with IFN- γ signaling, but this is also thought to interfere with antiviral state in the CNS allowing neurotropic viruses such as WNV and TBEV to invade the brain. Socs1 levels were significantly reduced in Tlr-8 deficient mice, which resulted in increased expression of IFN-I inducible genes, Isg-56 and Irf-7;

protecting the deficient mice from a lethal WNV challenge compared to WT mice (Paul and others 2016). Socs1 was identified to coprecipitate with Tlr-8, and short interfering RNA (siRNA) of Socs1 in RAW264.7 cell lines resulted in reduced expression of Tlr7 and Isg-56. The observation suggested that Socs1 could interact with Tlr-8 to interrupt antiviral immunity by reducing the expression of Tlr7 and hence a reduction in Tlr-7-induced antiviral molecules such as Isg-56 (Paul and others 2016).

Exploiting SOCS function via adaptive immunity

The complete “armory” of the immune system must be activated to fully overcome most viruses, and antibodies produced against viral epitopes forms an essential component in the antiviral arsenals. As described earlier, CD4+ T-helper cells function cooperatively with the B cells for efficient antibody production by B cells. Antibodies initially produced by B cells are immunoglobulin M (IgM) and for the efficient function, there must be a class switching recombination to IgA, IgE, or IgG, which are required to fight invading viruses at the mucosal surfaces. T cells activate B cells to class-switch (Nurieva and Chung 2010) via CD154 (CD40L) and cytokines, which can be interfered with IκBα and SOCS proteins (Qiao and others 2006). Qiao and others (2006) have demonstrated that HIV Nef protein accumulates in B cells *in vivo* and *in vitro* and interferes with B cells class switching by inducing IκBα, SOCS1, and SOCS3 to inhibit CD154 and cytokine signaling via NFκB pathway, making HIV-1 evade the total IgG and IgA immune response.

Protein inhibitors of activated STAT

The PIAS family proteins are SUMO-E3 ligases that form part of the sumoylation machinery. Sumoylation is a complex process of posttranslational modification involving many steps and component proteins that eventually results in attaching SUMO (as mono or poly-SUMO molecules) to lysine residues resident in SUMO motifs of target proteins. Some viruses benefit from a global sumoylated state while others find a global de-sumoylated cellular state favorable for viral replication (Lowrey and others 2017). A better understanding of how viruses modulate the global sumoylation process and various components of the machinery will prove beneficial in finding therapeutic targets to treat viral infections.

Viruses can modulate the cellular sumoylation processes to benefit viral persistence and pathogenesis (Lowrey and others 2017). Conn and others (2016) have demonstrated that PIAS4 levels are elevated in HSV-1 infection. Also, they observed that PIAS4 relocates to nuclear domains of viral replication to augment the antiviral effects of intrinsic promyelocytic leukemia (PML) protein. Depleting PIAS4 alone or in combination with PML interfered with the antiviral immune response against HSV-1 infection. Using human foreskin fibroblast (HF) cell line, Brown and others (2016) demonstrated that PIAS1 colocalizes with PML-nuclear bodies (PML-NB)—an intrinsic antiviral restriction factor. PML-NB acts as a scaffold or adapter protein by recruiting other constituent antiviral proteins such as Daxx and Sp100 to restrict a diverse range of viruses. PIAS1 is a

constituent protein in PML-NB formation and localizes with other PML-NB constituent proteins in focal nuclear domains with infecting HSV1 to restrict viral replication in a SUMO-dependent manner. However, with the expression of viral infected cell protein 0 (ICP0) (a viral protein that counteracts intrinsic antiviral immunity), PML levels are significantly reduced ($P < 0.001$) as it is gradually degraded via a proteasome that causes the dispersal of PIAS1 from nuclear domains with infecting HSV1 genomes (Brown and others 2016). Double knockdown of PIAS1 and PIAS4 upregulated replication of ICP0-null HSV1 in HF cells, suggesting a cooperative restriction activity of PIAS1 and PIAS4 (Brown and others 2016).

Parvovirus B19 is a known etiologic agent in inflammatory cardiomyopathy and parvovirus B19 infection or expression of the virus NS1 protein in HMEC-1 cells results in the upregulation of PIAS3 and STAT3. Upregulated STAT3/PIAS3 was associated with elevated expression in proteins involved in immune response such as IFNAR1, but a decrease in expression of antiviral molecules such as 2',5'-oligoadenylate synthetase 1 (OAS1) was observed. The dysregulated STAT/PIAS signaling is thought to contribute to B19-associated cardiomyopathy (Duechting and others 2008).

Vesicular stomatitis virus (VSV) and encephalomyocarditis virus (EMCV) induces higher expression levels of type-I IFNs (IFN α/β) in PIAS4^{-/-} than in PIAS4^{+/+} (competent) mouse embryonic fibroblasts (MEF) cells. Consequently, viral titers (of VSV and EMCV) are reduced in a time course manner in the PIAS4^{-/-} cells, suggesting a strong antiviral response mounted by high levels of type I IFN in the MEF cells (Kubota and others 2011). Kubota and others (2011) also demonstrated that PIAS4 inhibits type I IFN promoter activity in a SUMO-independent mechanism. This was evident in the reduction in expression levels of ISG15 and IFI56 with no interference with phosphorylation or nuclear translocation of IRF3 and IRF7, which are involved in TLR and RLR sensing and signaling of RNA viruses VSV and EMCV.

Relapse of HCV infection (only about 50% of people on IFN- α /ribavirin treatment respond to therapy) is associated with increased levels of PIAS3 in liver biopsies of 26 HCV patients suggesting that HCV manipulates the cell sumoylation machinery to promote viral replication (Ehrmann and others 2006). PIAS1 is also reportedly elevated in liver tissues of 33 patients who are nonresponsive to HCV therapy (El-Saadany and others 2013). And colleagues report similar findings of robust expression of PIAS1 in liver biopsies of HCV nonresponders and suggest that this could be a predictive marker of nonresponse to IFN- α /ribavirin combined therapy in HCV (Bautista and others 2007). PIAS2 has been reported to reduce the expression of key HCV proteins (HCV core, NS3, and NS5A) and also decrease viral assembly and budding, but did not necessarily reduce viral replication (Guo and others 2017).

While some viruses cause upregulation in the levels of PIAS proteins, others downregulate or target PIAS proteins for degradation to promote viral replication. Human papilloma virus E6 targets PIAS4 to inhibit cellular senescence (an antitumor mechanism used by mammalian cells) (Bischof and others 2006). Likewise, human cytomegalovirus hijacks PIAS1 from sumoylating viral immediate-early (IE) protein, which abrogates proteasomal degradation of viral IE

protein. As a result, there is reduced sumoylation of IE with a corresponding downregulation in the expression of ISGs, which eventually favors viral replication and propagation (Kim and others 2014). Adenovirus targets PIAS3 and re-localizes it to viral replication domains to ensure sumoylating and activating viral proteins and resulting in continued replication; hence interfering with the antiviral PML nuclear bodies formation and evading innate immune response (Higginbotham and O'Shea 2015).

Although PIAS restricts viral replication, ICP0 protein of HSV-1 can cause the relocalization of PIAS1 away from viral replicating centers and hence promoting lytic viral replication (Brown and others 2016). Ebola Zaire virus (EBOV) causes severe hemorrhagic disease in humans with case fatalities of about 90% in recent outbreaks in Africa. EBOV can interfere with IFN signaling, but the infection is characterized by spurious production of proinflammatory cytokines. EBOV VP35 has been shown by a yeast-2-hybrid system to interact with ubc9 and PIAS1 (both sumoylation machinery proteins) to increase the sumoylation of IRF7, which then repressed the transcription of IFNs. However, NF- κ B pathway which is involved in the spurious production of proinflammatory cytokines was not affected (Chang and others 2009). Although not a PIAS protein, nuclear pore protein—RanBP2, a SUMO E3 ligase (similar to PIAS proteins) is upregulated during JEV infection, and it is thought to play a role in restricting viral replication as downregulation of RanBP2, enhanced viral replication (Zhang and others 2013).

By far, the most spectacular way viruses modulate the sumoylation process is to make their own SUMO ligase as is the case in adenoviruses, Kaposi sarcoma-associated herpesvirus (KSHV), and foot and mouth disease virus (Chang and others 2010; Pennella and others 2010; Zhu and others 2015). Adenovirus E1-B55dDa protein is known to function as SUMO E3 ligase to sumoylate p53 and then shuttles it out of the nucleus into the cytoplasm for proteasomal degradation. It is believed that this is the strategy utilized by adenoviruses for cell transformation and tumorigenesis (Pennella and others 2010). KbZIP, a protein encoded by KSHV has a SIM and functions as a SUMO ligase, first sumoylating itself and then sumoylating target proteins such as p53 (tumor suppressor protein) by specifically attaching SUMO-2/3 to activate p53 and eventually inducing cell cycle arrest (Chang and others 2010). SOCS and PIAS expression kinetics in viral infections are summarized in Table 1.

SOCS and PIAS As Therapeutic Targets

Approach in cancer therapy

The potential of SOCS and PIAS as a therapeutic target in cancers has received some attention and has been explored (Shen and others 2004; Brantley and others 2008; Sugase and others 2017), but less attention has been given to viral infections. Since there is a disparity in the expression levels of SOCS in different cancers, experimented SOCS-targeted therapy has been 2-fold; (i) overexpression of SOCS levels to downregulate constitutively activated STATs that promotes cell growth and proliferation associated with cancers or (ii) suppression of SOCS in DC and T cells to activate STATs and enhance antitumor immunity (Jiang and others 2017). The potential of SOCS proteins as therapeutic targets

in cancer (which is outside the scope of our discussion) has been reviewed by Jiang and others (2017) and Mahony and others (2016).

Approach in viral therapy

SOCS and PIAS could serve as alternative therapeutic targets in flaviviral infections and to be able to do this efficiently, we need to appreciate more the landscape of using small molecules (peptidomimetics/inhibitors to bind and interfere with SOCS and PIAS function) and/or RNA interference (Worst and others 2016) with SOCS and PIAS as targets as have been explored in some cancers and antiviral therapy. Peptidomimetics are small synthesized peptides made to mimic the effect of a natural peptide or function similar to a specific motif in a protein (Vagner and others 2008) and about 60 of such peptides have been approved as drugs for human use (Qvit and others 2017). MicroRNAs (miRNAs) and siRNAs make up the RNA interference (RNAi) system and are short (about 19–25 nucleotides long) noncoding RNAs that can bind to complementary sequences in target mRNA to degrade, silence, and modulate the function of specific mRNAs. Very recently, the very first RNAi-based therapy (ONPATRO™) was approved by the United States Food and Drug Administration for the treatment of polyneuropathy caused by hereditary transthyretin-mediated amyloidosis in humans (Weng and others 2019). Studies focusing on SOCS and PIAS as therapeutic targets will serve as an alternative to some already on-going rational design of antiviral therapies. Comparatively, there have been a lot more studies done on SOCS than PIAS proteins.

Using antisense oligonucleotide technology (very similar to RNAi) to knock down SOCS3 expression in multiple human cell lines resulted in increased STAT activation and signaling and significantly reduced HSV-1 replication by about 10³ plaque forming unit/mL (Yokota and others 2005). HSV-1 induced SOCS1 in keratinocytes and this reduced STAT1 activation to promote viral replication in HEL30 keratinocytes. A SOCS1 antagonist, pJAK2 (a peptidomimetic) designed to mimic the activation loop of JAK2 binds the KIR domain of SOCS1 to interfere with SOCS1 inhibition of STAT1 phosphorylation to enhance IFN- γ signaling. pJAK2 at 35 μ M offered HEL30 keratinocytes about 40% protection from HSV-1 infection. pJAK2 at 35 μ M acted synergistically with 100 U/mL of IFN- γ to offer 100% protection from HSV-1 infection. Protection from HSV-1 infection by the peptidomimetic pJAK2 seems to be dose-dependent as 17 and 8 μ M offered 70% and 25% protection from HSV-1 infection, respectively (Frey and others 2009). The same peptidomimetic pJAK2, able to bind the KIR domain of SOCS1 and resulted in enhanced activity of IFN- γ and IL-6, with the consequent effect of mounting strong antiviral defense against lethal doses of HSV-1 and vaccinia virus to increase survival in mice models (Ahmed and others 2010, 2015b). pJAK2 has also been used to confer protection in mice against lethal doses of Influenza A virus (Ahmed and others 2015a).

miRNA-19a has been demonstrated to significantly reduce mRNA levels of SOCS1 and SOCS3 and consequently enhance IFN- α and IL-6 signaling through STAT3 to diminish the replication of both HCV and HBV in cell culture infection models (Collins and others 2013). Also, miRNA-122 can downregulate the expression of SOCS3 to enhance type I IFN

TABLE 1. *SOCs* AND *PIAS* EXPRESSION DYNAMICS AND MECHANISMS IN FLAVIVIRAL INFECTIONS

<i>Gene</i>	<i>Cell type</i>	<i>Organism</i>	<i>Expression</i>	<i>Function</i>	<i>Target proteins</i>	<i>Affected signaling pathway</i>	<i>Effect on virus</i>	<i>References</i>
<i>SOCs1</i>	Embryonic kidney cells (HEK293T), splenocytes	Human	Upregulation	Proteasomal degradation of JAKs	JAKs	JAK/STAT	ND	Vuong and others (2004)
	Macrophages	Mouse	Upregulation	Compete with STAT1 phosphorylation	STAT1	JAK/STAT	ND	Flowers and others (2004)
	Splenocytes embryonic fibroblast	Mouse	Upregulation	Enhance proteolysis of p65 subunit of NF- κ B	NF- κ B	NF- κ B	ND	Ryo and others (2003)
	HEK293T	Human	Upregulation	Degrade MAL	MAL, IRAK	NF- κ B	ND	Mansell and others (2006)
	Macrophages	Human	Upregulation	Increased degradation of pSTAT2	STAT2	JAK/STAT	Increased DENV replication	Estrada-Jimenez and others (2016)
	Macrophages	Human	Upregulation	Reduced nuclear import of pSTAT1	STAT1	JAK/STAT	Increased DENV replication	Estrada-Jimenez and others (2016)
	Macrophages	Mouse	Upregulation	Down-regulate IFN- α	IFN- α	JAK/STAT	Increased JEV replication	Kundu and others (2013)
<i>SOCs2</i>	Macrophages	Mouse	Upregulation	Reduced phosphorylation of STAT1/2	STAT1/2	JAK/STAT	Increased JEV replication	Kundu and others (2013)
	Cervical epithelia	Human	Upregulation	Interferes with IFN- λ signaling		IFN- λ	Increased DENV replication	Palma-Ocampo and others (2015)
	Macrophages	Mouse	Upregulation	Downregulate Tlr-7 expression	Tlr8	JAK/STAT	Increased WNV replication	Paul and others (2016)
<i>SOCs3</i>	Neural cells	Mouse	Upregulation	Not defined	JAKs	JAK/STAT	Increased WNV and TBEV replication	Mansfield and others (2010)
	IFNAR ^{-/-} HEK293T	Mouse & Human	Upregulation	Interferes with STAT1 binding to IFN receptors	STAT1	JAK/STAT	Increased Semliki Forest Virus (SFV) replication	Fenner and others (2006)
	Macrophage	Mouse	Upregulation	Compete with STAT1 phosphorylation	STAT1	JAK/STAT	ND	Flowers and others (2004)
<i>CIS</i>	Macrophages	Mouse	Upregulation	Downregulate IFN- α	IFN- α	JAK/STAT	Increased JEV replication	Kundu and others (2013)
	Macrophages	Mouse	Upregulation	Reduced phosphorylation of STAT1/2	STAT1/2	JAK/STAT	Increased JEV replication	Kundu and others (2013)
	Cervical epithelia	Human	Upregulation	Interferes with IFN- λ signaling		IFN- λ	Increased DENV replication	Palma-Ocampo and others (2015)
<i>PIAS1</i>	Neural cells	Mouse	Upregulation	Not defined	JAKs	JAK/STAT	Increased WNV and TBEV replication	Mansfield and others (2010)
	Hepatocytes	Human	Upregulation	Interferes with STAT1 and 2 activation	STAT1	JAK/STAT	Increased RSV replication	Hashimoto and others (2009)
	Epithelia (U3A cells)	Human	Upregulation	Inhibit DNA-binding activity of STAT1	STAT1	JAK/STAT	ND	Liu and others (1998)
<i>PIAS3</i>	Embryonic kidney cells (HEK293T)	Human	Exogenous	Repress transcriptional activity of STAT1 and NF- κ B		JAK/STAT, NF- κ B	ND	Liu and others (2005)
	Cervical epithelia	Human	Exogenous	Inhibits DNA-binding activity of IRF3	IRF3	Type I IFN signaling	ND	Li and others (2013)
	Embryonic kidney cells (HEK293T)	Human	Exogenous	Bind p65 subunit to downregulate NF- κ B modulated genes	NF- κ B	NF- κ B	ND	Liu and others (2005)
<i>PIAS4</i>	Hepatocytes	Human	Upregulation	Prevents DNA binding by STAT3	STAT3	JAK/STAT	ND	Chung and others (1997)
	HEK293T	Human	Exogenous	Sequesters LEF1	LEF1	Wnt signaling	ND	Sachdev and others (2001)
	Fibroblast	Human	Upregulation	Antiviral factor	Viral DNA	Not defined	Decreased HSV replication	Conn and others (2016)

RSV, respiratory syncytial virus; ND, not determined; *SOCs*, suppressors of cytokine signaling; *CIS*, cytokine-inducible SH2-containing protein; *PIAS*, protein inhibitors of activated STAT; STAT, signal transducer and activator of transcription; JAK, Janus kinase; NF- κ B, nuclear factor kappa-light-chain enhancer of activated B cells; MAL, myelin and lymphocytes protein; IRAK, interleukin receptor activating kinase; IFN, interferon; IRF, IFN regulatory factor; WNV, West Nile virus; DENV, dengue virus; JEV, Japanese encephalitis virus; TBEV, tick-borne encephalitis virus; HSV, herpes simplex virus.

activity against HBV replication in Huh7 cells (Gao and others 2015). siRNA-mediated knockdown of PIAS2 has also been shown to significantly reduce HBV gene expression and viral replication in HEK293T cells (Pan and others 2017). Complementary sequences for miRNA-221 have been discovered in the 3'UTR of SOCS1 and SOCS3 and hence miRNA221 can downregulate the expression of SOCS1 and SOCS3 resulting in about 2-fold increase in the phosphorylation levels of JAK1, STAT1, and STAT3 to eventually enhance the antiviral effect of IFN- α on HCV replication.

IFN- α /ribavirin combined administration used to be the treatment of choice in HCV infection until the introduction of directly acting antivirals against HCV. Elevated levels of miRNA221 in the serum of HCV-infected patients have therefore been proposed as one of the mechanisms that accentuate the antiviral effect of IFN- α treatment success in HCV patients by downregulating the expression of SOCS1 and SOCS3 (Xu and others 2014). Viral-induced upregulation of SOCS3 and PIAS1 is involved in HCV resistance to IFN- α and ribavirin therapy, whereas patients who respond to therapy have significantly reduced levels of SOCS3 and PIAS1 (El-Saadany and others 2013). PIAS4 overexpression in Huh7 cells induces autophagy by increasing the expression and accumulation of SUMO1 conjugated proteins, but on the contrary, downregulating PIAS4 expression reduced HCV replication (Ran and others 2018). Strategies to reduce levels of SOCS3 and PIAS1 could, therefore, help minimize or eliminate HCV resistance to current therapy.

HIV-1 like other viruses are obligate parasites and hence depend heavily on host molecular systems to make virus progeny. It has been reported that HIV-1 induced-SOCS1 bind to Gag polypeptide of the virus to stabilize it and enhance the cellular trafficking of this protein within cells to viral replicating centers leading to higher HIV-1 titers. Knocking down SOCS1 by siRNA resulted in Gag accumulation in perinuclear centers and easily targeted for proteasomal degradation and this significantly inhibited viral replication, suggesting a promising therapeutic potential of SOCS1 in HIV-1 drug design approaches (Ryo and others 2008).

Li and others (2013) demonstrated using HEK293T cell lines that siRNA-mediated knockdown of endogenous PIAS1 potentiated IFN signaling when the cells were infected with Sendai virus resulting in higher levels of antiviral molecules (IFNB1, ISG56, and IL-8).

SOCS3 and its regulation of IFN signaling, Ras/extracellular signal-regulated kinase, and NF- κ B pathways are well characterized in many diseases (cancers, rheumatoid arthritis, and metabolic disorders) and infections (bacterial and viral), and the potential of SOCS3 as a therapeutic target has been reviewed recently (Mahony and others 2016; Pedroso and others 2018). Apart from the genetic approaches to downregulate the expression of SOCS3, zoledronic acid (ZA), a drug approved for the treatment of bone diseases such as osteoporosis and Paget's disease, has been applied to reduce the expression of SOCS3 in macrophages. Scheller and others (2011) observed that SOCS3 transcription is modulated by nuclear accumulation of pSTAT3, and the application of ZA resulted in a significant reduction in pSTAT3 and consequently reduced levels of SOCS3 in human macrophages.

JEV has been found to downregulate the levels of miRNA-432 (which is known to have complementary sequences in the 3'UTR of SOCS5), which then causes an increase in the expression levels of SOCS5 in CHME3 cell

lines as well as *in vivo* in mouse brain tissues (Sharma and others 2016). Overexpressing miRNA-432 in CHME3 cells and mice brain tissue led to the downregulation of SOCS5 and enhanced JAK/STAT signaling due to increasing levels of phosphorylated STAT1, IL-6, and TNF- α , and eventually suppressed JEV viral replication. In an alternative approach, siRNA-mediated knockdown SOCS5 resulted in increased levels of p-STAT1 and proinflammatory markers (IL-6 and TNF- α) to reduce JEV replication in infected cells by about 50% (Sharma and others 2016). Knocking down SOCS1 and SOCS3 in JEV-infected murine primary macrophages resulted in increased phosphorylation and activation of STAT1 and STAT2, leading to increased expression of type I IFNs and a concomitant expression of antiviral proteins such as OAS and IFIT1 with eventual significant reduction in viral replication (6-fold and 5-fold with SOCS1 and SOCS3 siRNA, respectively) (Kundu and others 2013).

Previously described approaches have focused on reducing viral replication, but hemorrhagic fever associated with some flaviviral infections might not necessarily be due to the viral load but the imbalance in T helper Th1 and Th2 immune responses (Chaturvedi and others 2000). Reduced levels of SOCS1 and increased levels of miRNA-150 (known to downregulate SOCS1) causes a shift from a Th1 to Th2 immune response and has been associated with dengue hemorrhagic fever (the severe form of dengue infection) (Chen and others 2014).

Altogether, this review has given a comprehensive exposition on the potential of SOCS and PIAS as therapeutic targets (with SOCS looking more promising than PIAS) and how previous studies have applied this to the design of therapy against some cancer and viral diseases. Considering promising results of pJAK2 (SOCS1 peptidomimetic) in mice studies against many viruses, it is plausible that it could be effective against other RNA viruses, including those in Flaviviridae, which remain a global threat.

Conclusion

Unfortunately, flaviviruses remain a significant global health burden with high levels of morbidity and mortality, but no successful antiviral therapies are available yet. As frantic efforts are made to develop vaccines against this family of viruses, we argue for the exploration of more alternative therapeutic approaches. With the knowledge that flaviviruses have used a number of mechanisms to evade host immune response, including exploiting SOCS and PIAS levels, rational approaches to modulate SOCS and PIAS expression levels through RNA interference or small molecule antagonist techniques have the potential to tilt the immune balance in favor of the host to overcome the onslaught of these viruses and to improve the survival of infected persons.

Authors' Contributions

M.B.Y. wrote the article. D.F. and O.Q. read and edited the article extensively. All authors reviewed and approved the article.

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