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Putative Roles of SOCS1: SOCS 3 Ratios in Development and Resolution of a Herpesvirus Lesion

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Abstract

This article is the author's opinion of the effects of the cytokines produced as a result of epithelial cell-macrophage interactions during herpes simplex virus type 1(HSV-1) infection. Cell cultures of murine keratinocytes were examined for SOCS1 production following HSV-1 infection and macrophage cell lines were examined for cytokine production following polarization and modification of cytokine production by peptide mimetics of suppressors of the cytokine signaling (SOCS) molecules SOCS1 and SOCS3. It is speculated that the pro inflammatory cytokines produced by M1 polarized cells enhance the inflammatory nature of the lesion produced by HSV-1 and the anti-inflammatory cytokines of the M2 polarized cells promote resolution of the lesion.

Summary

We previously noted that murine keratinocyte cell lines (HEL-301 and PAM-212) produced large amounts of SOCS1 mRNA and protein following infection with HSV-1 or treatment with interferon-gamma (IFN-γ) [1]. In contrast, murine fibroblasts (L929) exhibited minimal increase in SOCS1 levels when treated with IFN-γ following infection with HSV-1 [1]. This antiviral state was induced in fibroblasts but not in keratinocytes. The resistance of keratinocytes to IFN-γ correlated with the hyperinduction of SOCS1 in these cells [1].

During the first 24 hours of HSV-1 infection, changes in morphology, CD14-CD86 expression, cell viability, and SOCS protein levels were examined in macrophage cell lines (J774A.1 and RAW 264.7) [2]. We found that polarization treatments alone induced changes in morphology and viability of murine macrophage cell lines (J774A.1 and RAW 264.7). M1 macrophages, polarized by treatment with lipopolysaccharide (LPS) and interferon-gamma (IFN-γ) appeared flattened and vacuolated while M2 cells, polarized by treatment with interleukin (IL)-4, were elongated with few vacuoles. In contrast to unstimulated cells (M0), HSV-1 infection caused rounding of unpolarized or polarized M1 and M2 macrophages in cell culture at 24 hours after infection as well as decreases in expression of CD14 (LPS receptor) and CD86 [2]. M1 macrophages did not upregulate SOCS1 following virus challenge, however, SOCS3 levels increased [2].

Similar to our observations using M1 and M2 polarized murine macrophage cell lines [unpublished observation], Jaguin and colleagues [3] found that the mannose receptor CD206 did not distinguish between the M1 and M2 phenotypes of human macrophages. They used monocytes purified from the buffy coats of human peripheral blood cells to characterize phenotypic and genomic markers [3]. They generated macrophages from these primary human cells by treatment with macrophage colony stimulating factor (M-CSF), polarizing them using the same inducers discussed in the previous paragraph; cells were polarized to the M1 phenotype using LPS and IFN-γ or polarized to the M2 phenotype using IL-4. The cell membrane marker unique to M1 cells was CD80 (B7.1); we found CD86 (B7.2) unique to the M1 phenotype of J774A.1 macrophages [2]. CD200R expression (involved in down regulation of myeloid function) was unique to the M2 polarized human macrophages; we recently found CD200R expressed by RAW 264.7 macrophages polarized to the M2 phenotype with either IL-4 or IL-10 but not with IL-13 [Al Sharif S and Bigley NJ, unpublished observations].

HSV-1-infected unpolarized (M0) J774A.1 cells exhibited significant increases in expression levels of native SOCS1. Up regulation of SOCS3 expression in HSV-1-infected M1 macrophages over that seen in uninfected M1 cells may reflect the effects of M1 polarization or suggest the cell's attempt to counteract effects of pro inflammatory molecules [2]. Qasimi and colleagues showed that different domains of SOCS3 protein mediate interleukin-10 (IL-10) inhibition of TNF-α and nitric oxide production by this same macrophage cell line [4]. In this same macrophage cell line (J774A.1), IL-10 was responsible for the anti-inflammatory response to Borrelia burgdorferi [5]. In our macrophage cultures, SOCS1:SOCS3 expression levels appeared relatively unchanged in virus-infected M2 macrophages when compared to their uninfected counterparts, suggesting microenvironment signals such as IL-4 play a greater role in SOCS expression levels than does HSV-1 infection [2]. We then hypothesized that the HSV-1-infected J774A.1 or RAW264.7 M1 macrophages were attempting to counteract the effects of inflammatory molecules induced by polarization.

Jo and colleagues [6] used a recombinant cell-penetrating form of SOCS3 (CP-SOCS3) to protect mice (C3H/HeJ) from the lethal effects of SEB and LPS by reducing production of inflammatory cytokines and attenuating apoptosis and hemorrhagic necrosis. Within 2 hours after injection, CP-SOCS3 was distributed in multiple organs and persisted for at least 8 hours. The membrane-translocating motif (MTM) was composed of 12 amino acids from a hydrophobic signal sequence from fibroblast growth factor 4 [6]. The MTM was attached to either the N-terminal or C-terminal of SOCS3. Only these forms were capable of penetrating RAW cells. The peptide mimetics in this present study were provided by Dr HM Johnson and his colleagues at the University of Florida [7]. These peptides contain a lipophilic group (palmitoyl-

lysine) added to the N terminus of the synthetic peptide which provides them with the ability to penetrate cells. SOC S3 peptide mimetic and the SOC S1 inhibitor (pJAK2) increased the viability of polarized M1 cells over SOCS1 peptide mimetic-treated M1 J 774A.1 or RAW264.7 macrophages, similar to the observations in comparable cell groups infected with HSV-1 ($p<0.001$) [Al Sharif S and Bigley NJ, unpublished observations].

We predicted that the anti-inflammatory effect in these cells would be characterized by increased levels of IL-10. IL-10 predominated in supernatant fluids collected from macrophage cultures at 24 hour after M2 polarization [Al Sharif S and Bigley NJ, unpublished observations. Because addition of SOCS1 peptide mimetic decreased the viability of polarized M1 cells and HSV-1-infected M1 J774A.1 or RAW 264.7 macrophages ($p<0.001$), we predicted that the inflammatory effect in these cells would be characterized by increased levels of TNF-α. IL-6 and TNF-α were found in supernatant fluids collected at 24 hours after M1 polarization (Al Sharif S and Bigley NJ, unpublished observations). We also noted that SOCS3 peptide mimetic protects macrophages (RAW 264.7 and J774A.1) from the lytic effect of HSV-1 and from the lytic effect of M1 polarization. Benveniste's group previously established the anti-inflammatory role of SOCS3 in microglial (macrophage-like) cells in a murine model of multiple sclerosis [8-10].

Our observations made in HSV-1 infection of keratinocytes (diagrammed in the left panel of the figure below) led to the consideration of the effects of these infected epithelial cell on the underlying tissue macrophages as shown by our studies on polarized murine macrophage cell lines (right panel). Both HSV-1 and IFN-γ induced murine epithelial cell lines (HEL-30 and PAM 212) to produce SOCS1. When the epithelial cells were pretreated with a SOCS 1 antagonist (pJAK2), they became susceptible to the protective effect of IFN-γ and were resistant to HSV-1 infection. We suggest that the SOCS1 and HSV-1 produced during the lytic infection of the mucosal keratinocytes following HSV-1 infection directly affects the underlying tissue macrophages. The right panel of this figure summarizes our findings with polarized murine macrophage cell lines (RAW264.7 and J774A.1) [Al Sharif S and Bigley NJ, unpublished observations]. HSV-1 infection decreased the viability of M1 polarized RAW 264.7 macrophages significantly over that seen by polarization alone ($p<0.005$). The cytokine data summarized in the figure were obtained only from polarized cells and not from virus-infected polarized cells (yet to be determined). M1 polarized cells treated with SOCS3 peptide mimetic produced increased levels of the anti-inflammatory cytokine IL-10 and markedly decreased levels of the pro-inflammatory cytokines IL-6 and TNF-α as did macrophages polarized to the M2 phenotype. Conversely, M1 polarized macrophages treated with the SOCS1 peptide mimetic produced marked increases in the pro inflammatory cytokines (IL-6 and TNF-α) and much less IL-10, similar to polarized M1 cells.

As diagrammed in Figure 1, we suggest that the lesions formed by HSV-1 infection of mucosal/skin surfaces result from the M1 cytokine response heightened by the virus. Resolution of such lesions would result when the ratio of SOCS1: SOCS3 shifts so that SOCS3 levels are increased. Factors triggering this shift in SOCS ratios are unknown but likely represent other attributes of innate immunity signaling pathways, e.g., Toll-like receptors (TLRs) and viral nucleic acid sensors, involved in cross-talk between epithelial cells and underlying monocytes/macrophages.

The observations of Wang et al [11] with Kupffer cells, resident liver macrophages, support our finding that IL-10 predominates in the M2 macrophage expressing higher levels of SOCS3 than SOCS1. In most cells SOCS3 is associated with STAT3 activation following IL-6 ligation. In macrophages IL-6 ligation with IL-6R and gp130, expressed at high levels on Kupffer cells, leads to transient activation of STAT3. Kupffer cells express high levels of both IL-10R1 and IL-10R2 and when ligated with IL-10 prolonged activation of STAT 3 results. STAT3 activation induces expression of SOCS3, which in turn inhibits IL-6 activation of STAT3, but does not inhibit IL-10 signaling.

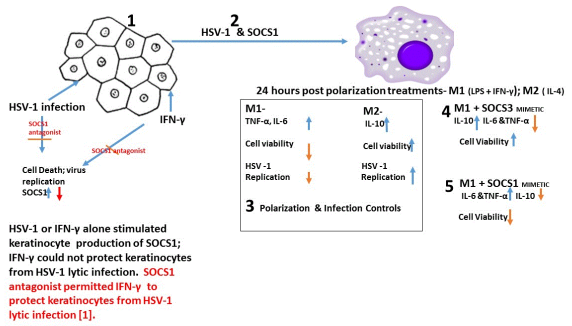

Figure 1: Putative interactions between HSV-1-infected epithelial cells and mucosal macrophages. 1. Keratinocytes, the initial cells infected in HSV-1 pathogenesis, allow viral replication and express SOCS1. Virus and SOCS proteins are released by lysis of infected cells. This process can be alleviated by treatment of keratinocytes with inhibitor of SOCS1. 2. Tissue monocytes/macrophages in underlying mucosa respond to released virus and cell debris by secreting SOCS molecules. 3. Cultures of macrophages (J774A.1 or RAW 264.7) stimulated by SOCS 1 produce pro inflammatory cytokines (IL-6, TNF-α), exhibit decreased viability, and release 3.5-4 fold less virus than infected, unpolarized cells. By comparison, HSV-1-infected macrophages polarized to the M2 phenotype produce anti-inflammatory IL-10 and exhibit enhanced cell viability and increased virus replication. 4. Polarized M1 macrophage responses can be manipulated to resemble the M2 phenotype by treatment with SOCS3 peptide mimetic; these cells show enhanced production of IL-10, increased viability compared with M1 phenotype and increased capacity to replicate virus. 5. Polarization of the M1 macrophage phenotype is maintained by treatment with SOCS1 peptide mimetics as shown by increased production of pro inflammatory cytokines (IL-6,TNF-α), increased cell viability compared to M1cells and deceased ability to replicate virus.

Consistent with our observations with epithelial cells, Yu and colleagues noted that overexpression of SOCS1 in transgenic rat eyes alleviated subsequent ocular HSV-1 infection [12]. This same group recently observed that a 16 amino acid SOCS1 mimetic inhibited expansion of TH17 inflammatory cells in a mouse model of experimental autoimmune uveitis [13], indicating the potential of using SOCS peptide mimetics in manipulating inflammatory processes initiated by insults to epithelial cells. Further study of SOCS1:SOCS3 ratios and relative JAK/STAT signaling pathways in the responding macrophage population is suggested by our observations.

In support of our contention that SOCS proteins can be released from cells are the observations of Bourdonnay and colleagues [14]. They found that alveolar macrophages secreted SOCS1 and SOCS3 in exosomes and microparticles which were then taken up by alveolar

epithelial cells and subsequently inhibited activation of STAT [14]. In our model of lesion formation (Figure 1), we suggest that virus-infected epithelial cells (keratinocytes) secreted SOCS1 which then influenced the underlying macrophages. The observations of Bourdonnay's group also provide an explanation for the resolution of HSV-1 epithelial lesions mediated by the inhibition of inflammatory JAK/STAT pathways in uninfected epithelial cells and macrophages following micropinocytosis of SOCS1- and SOCS3 -rich microparticles released from polarized/activated macrophages.

Our studies with HSV-1 infected murine macrophage cell lines fit well with Ellermann-Eriksen's model for the role of macrophages in the early host response to HSV-1 infection [15]. He contended that during the early hours of herpes virus infection, Type I interferon and tumor necrosis factor (TNF) cytokines are secreted and exhibit anti-virus and macrophages- activating behaviors. Activated macrophages then secrete IL-12 which activates natural killer (NK) cells to produce IFN-γ which activates macrophages to produce reactive oxygen species (ROS) and nitric oxide (NO). The early macrophage responses described by Ellermann-Eriksen [15] are compatible with the M1 phenotype we have observed.

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References

1. Frey KG, Ahmed CM, Dabelic R, Jager LD, Noon-Song EN, et al. (2009) HSV-1-induced SOCS-1 expression in keratinocytes: use of a SOCS-1 antagonist to block a novel mechanism of viral immune evasion. J Immunol 183: 1253-1262.
2. Reichard AC, Cheemarla NR, Bigley NJ (2015) SOCS1/3 expression levels in HSV-1-infected, cytokine-polarized and -unpolarized macrophages. J Interferon Cytokine Res 35: 32-41.
3. Jaguin M, Houlbert N, Fardel O, Lecureur V (2013) Polarization profiles of human M-CSF-generated macrophages and comparison of M1-markers in classically activated macrophages from GM-CSF and M-CSF origin. Cell Immunol 281: 51-61.
4. Qasimi P, Ming-Lum A, Ghanipour A, Ong CJ, Cox ME, et al. (2006) Divergent mechanisms utilized by SOCS3 to mediate interleukin-10 inhibition of tumor necrosis factor alpha and nitric oxide production by macrophages. J Biol Chem 281: 6316-6324.
5. Dennis VA, Jefferson A, Singh SR, Ganapamo F, Philipp MT (2006) Interleukin-10 anti-inflammatory response to Borrelia burgdorferi, the agent of Lyme disease: a possible role for suppressors of cytokine signaling 1 and 3. Infect Immun 74: 5780-5789.
6. Jo D, Liu D, Yao S, Collins RD, Hawiger J (2005) Intracellular protein therapy with SOCS3 inhibits inflammation and apoptosis. Nat Med 11: 892-898.
7. Ahmed CM, Dabelic R, Waiboci LW, Jager, LD, Heron, LL, et al. (2009). SOCS-1 mimetics protect mice against lethal poxvirus infection: Identification of a novel endogenous antiviral system. J Virol 83: 1402-1415.
8. Akhtar LN, Benveniste EN (2011) Viral exploitation of host SOCS protein functions. J Virol 85: 1912-1921.
9. Qin H, Yeh W, De Sarno P, Holdbrooks AT, Liu Y, et al. (2012) Signal transducer and activator of transcription-3/suppressor of cytokine signaling-3 (STAT3/SOCS3) axis in myeloid cells regulates neuroinflammation. Proc Natl Acad Sci U S A 109: 5004-5009.
10. Qin H, Holdbrooks AT, Liu Y, Reynolds SL, Yanagisawa LL, et al. (2012) SOCS3 deficiency promotes M1 macrophage polarization and inflammation. J Immunol 189: 3439-3448.
11. Wang H, Lafdil F, Kong X, Gao B (2011) Signal transducer and activator of transcription 3 in liver diseases: a novel therapeutic target. Int J Biol Sci 7: 536-550.
12. Yu CR, Hayashi K, Lee YS, Mahdi RM, Shen de F, et al. (2015) Suppressor of cytokine signaling 1 (SOCS1) mitigates anterior uveitis and confers protection against ocular HSV-1 infection. Inflammation 38: 555-565.
13. He C, Yu C-R, Sun L, Mahdi RM, Larkin III, et al. (2015) Topical administration of a suppressor of cytokine signaling-1 (SOCS1) mimetic peptide inhibits ocular inflammation and mitigates ocular pathology during mouse uveitis. J Autoimm 62: 31-38.
14. Bourdonnay E, ZasÅ,ona Z, Penke LR, Speth JM, Schneider DJ, et al. (2015) Transcellular delivery of vesicular SOCS proteins from macrophages to epithelial cells blunts inflammatory signaling. J Exp Med 212: 729-742.
15. Ellermann-Eriksen S (2005) Macrophages and cytokines in the early defence against herpes simplex virus. Virol J 2: 59.