Differential Expression of SOCS1/SOCS3 Ratios in Virus-Infected Macrophage Cell Lines

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We previously noted that murine keratinocyte cell lines (HEL-301 7 PAM-212) RODUCED large amounts of SOCS1 mRNA and protein following infection with SV-1 or treatment with interferon-gamma (IFN-γ). In contrast, murine fibroblasts 929) exhibited minimal increase in SOCS1 levels when treated with IFN-γ llowing infection with HSV-1 (Frey et al. 2009).

n antiviral state was induced in fibroblasts but not in keratinocytes. This esistance of keratinocytes to IFN-γ corresponded to the hyperinduction of SOCS1 these cells.

he goal of the present study was to determine the effects of HSV-1 infection on orphology, CD14-CD86 expression, cell viability, and SOCS protein levels in olarized M1 and M2 macrophage cell lines (J774A.1 and RAW 264.7) during the rst 24 hour of infection. For comparison we examined these responses against he monocyte-macrophage trophic Dengue virus (DENV2) in the RAW 264.7).

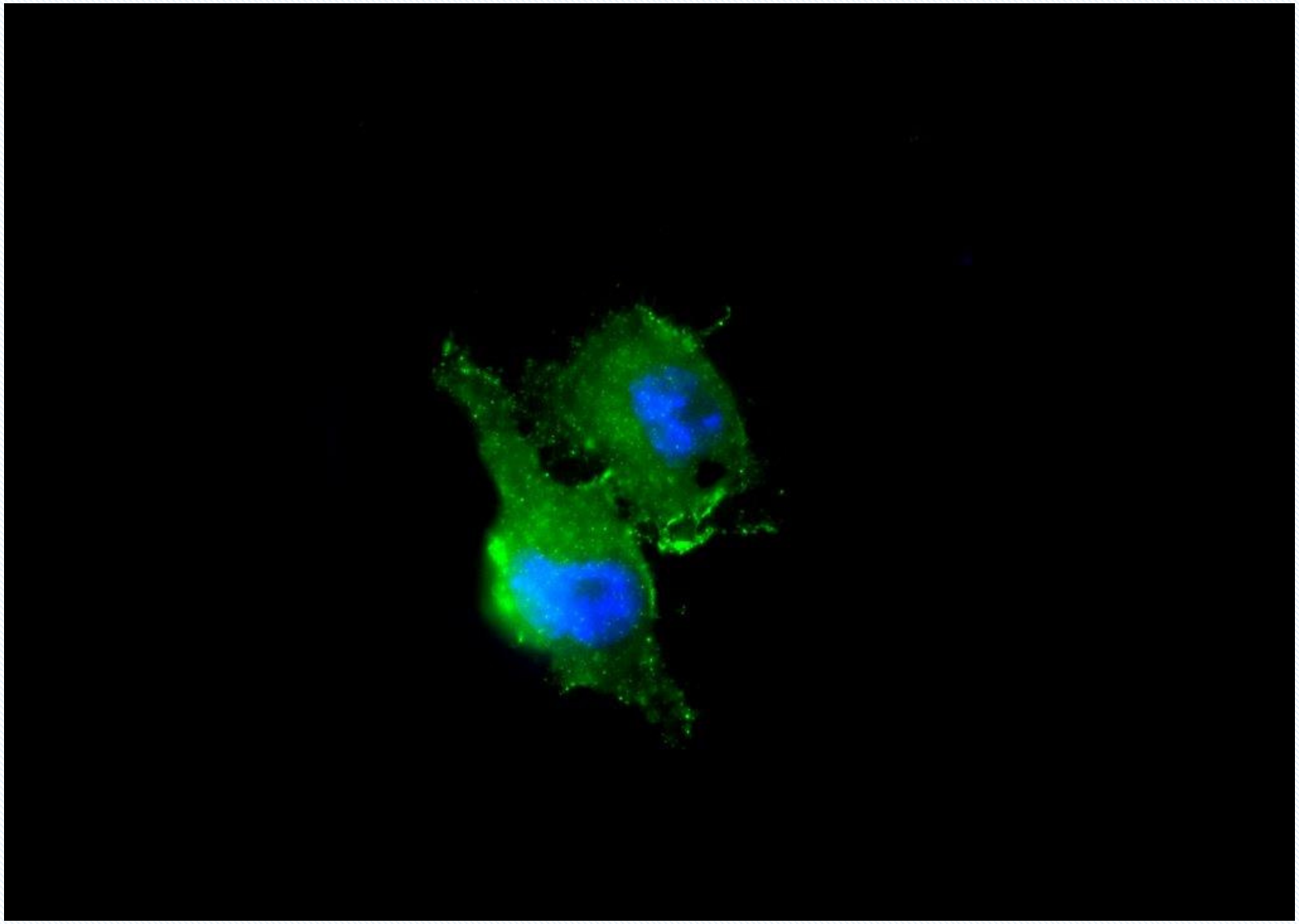
Viruses

rpes Simplex Virus-1 strain Syn 17+ (HSV-1) initially obtained from Dr. Nancy Sawtell, Children's Hospital edical Center, Cincinnati, OH was propagated on confluent monolayers of Vero cells. After 4-5 days post ection or when CPE was evident, the cells were spun down, supernatant was aliquoted and stored at -°C. Virus was quantified by infecting Vero cell monolayers with different dilutions of virus and plaque ming units were counted to calculate volume required for 0.1 multiplicity of infection (MOI).

ngue Virus DENV serotype 2 (DENV-2) was provided by Dr. Eric M. Vela, Battelle Memorial Institute search Center. DENV2 was propagated on Vero 76 cells. Briefly, Vero 76 cells grown in 100 mm petri hes to a confluence of approximately 85% at 37 °C, were infected with DENV-2 for 5-6 days or until CPE s evident. Cells were then scraped and centrifuged at 1500 rpm to eliminate cell debris. The supernatant d was aliquoted and stored at −80°C until use. Dengue virus titers were determined by plaque assay on nfluent monolayers of Vero 76 cells grown in 6-well plates

Murine Macrophage Cell Lines

4.7 (ATCC TIB-71) and J774A.1 (TIB-67) cells lines were obtained from the American Type Culture llection (ATCC) Manassas, VA.



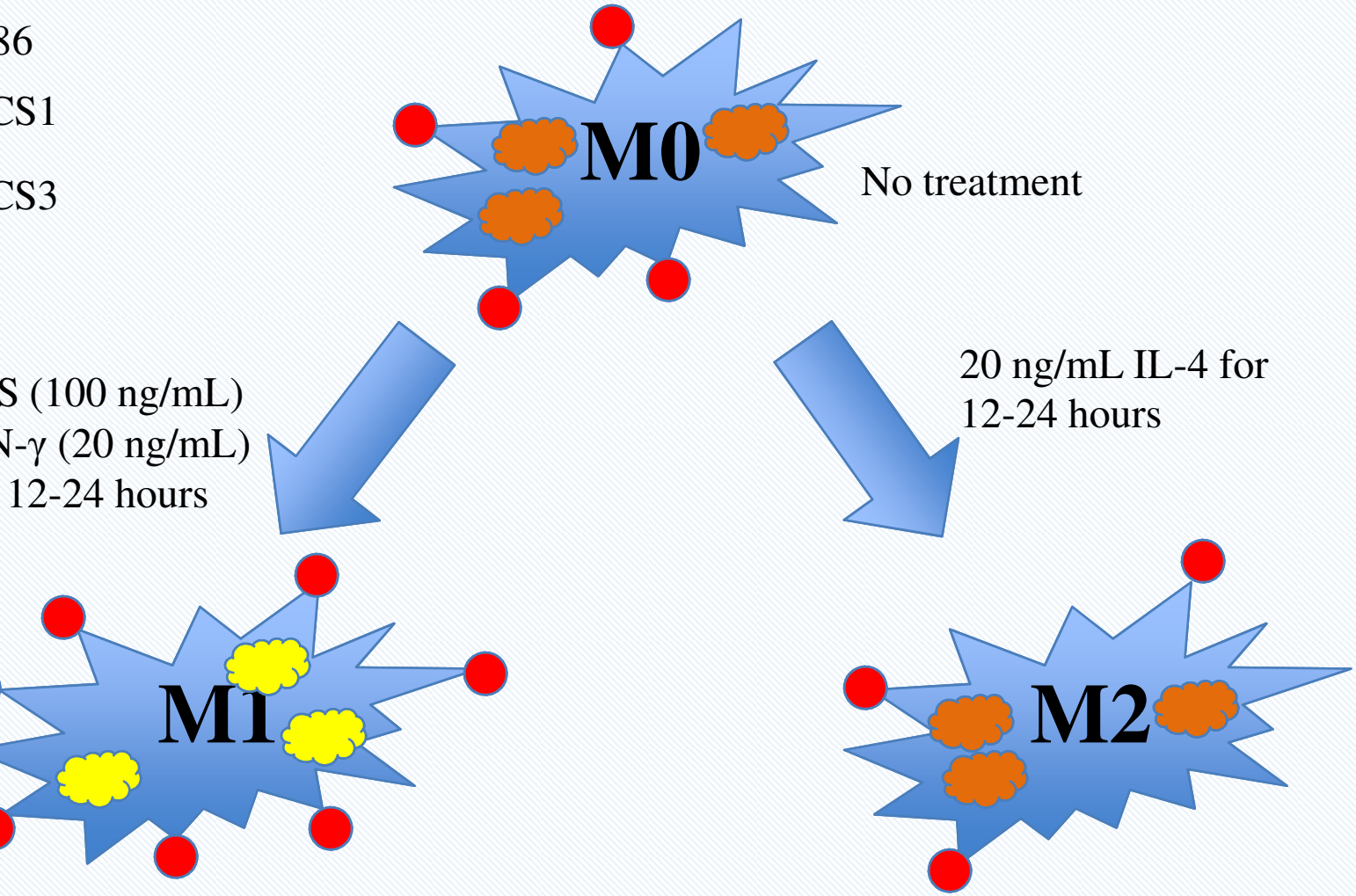
DENV2 infection of RAW 264.7 macrophages at 3 days post infection

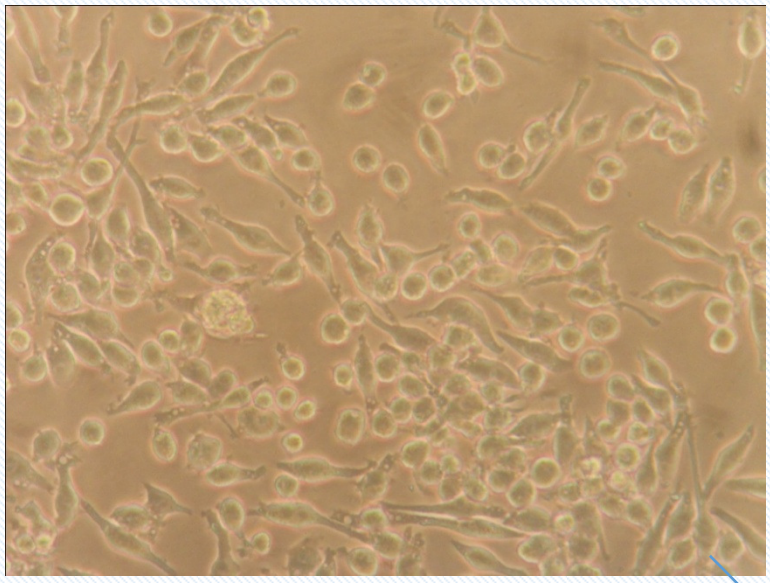
Macrophage Polarization Treatment

86

CS1

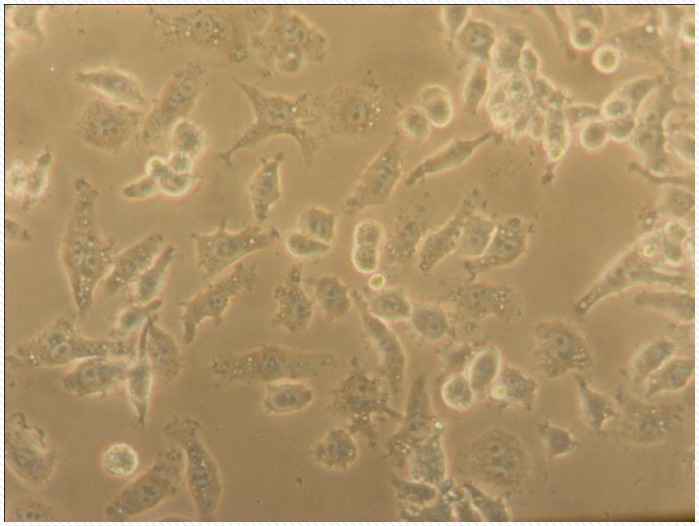
CS3

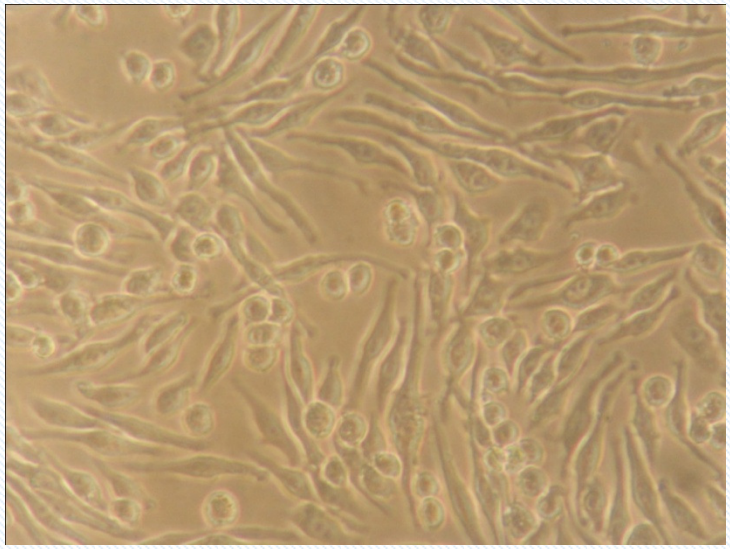




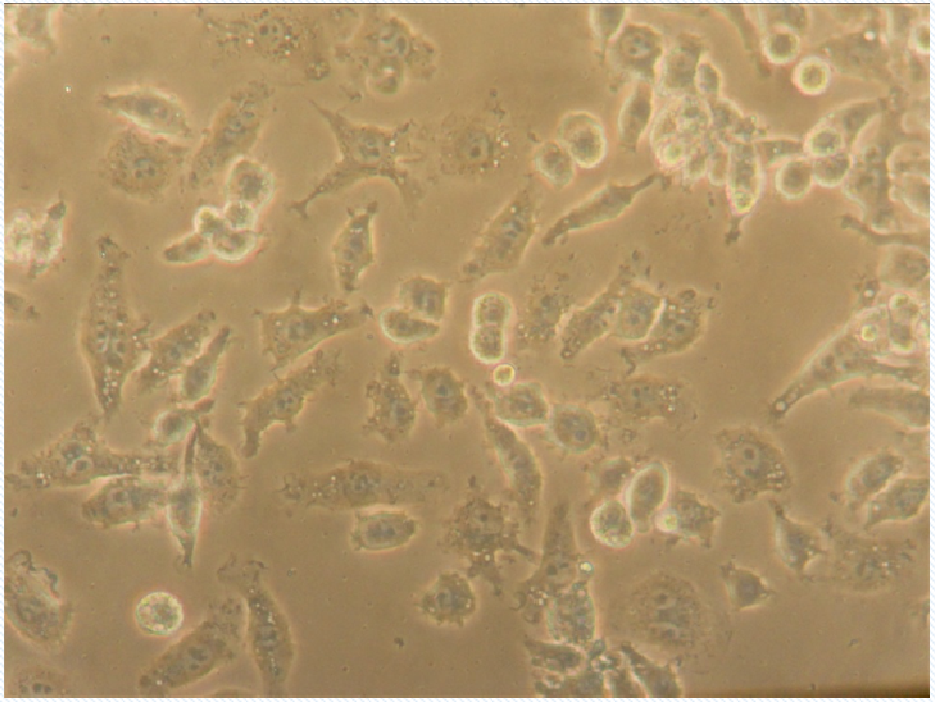
LPS &
IFN-γ

IL-4

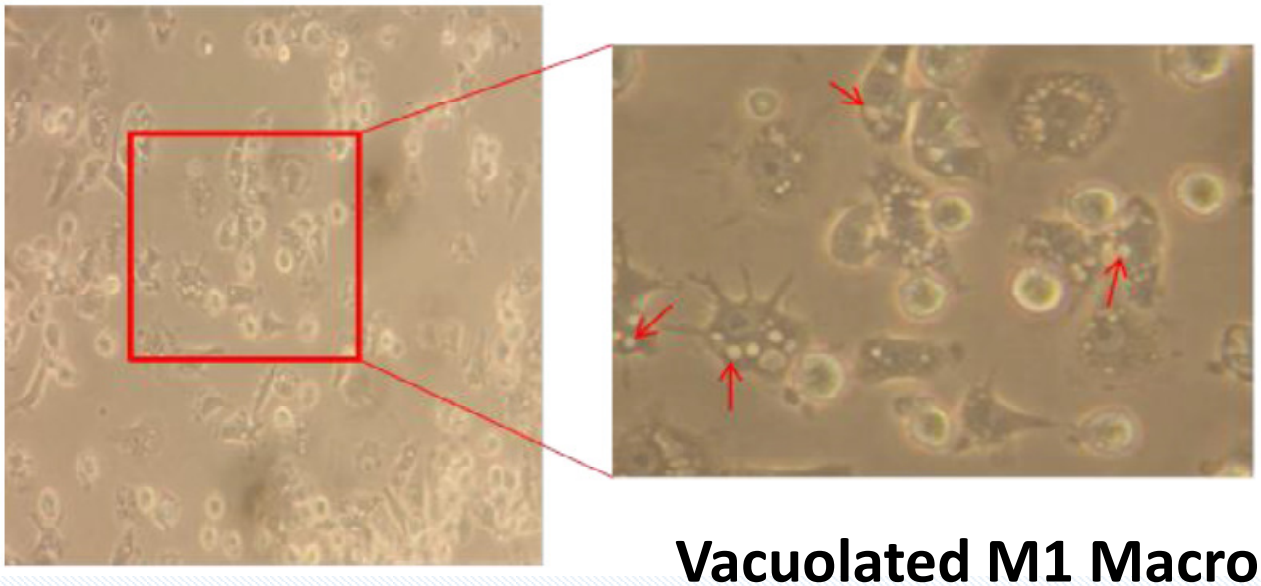




J774A.1 Macrophages at 24 hours after polarization



M1 J774A.1

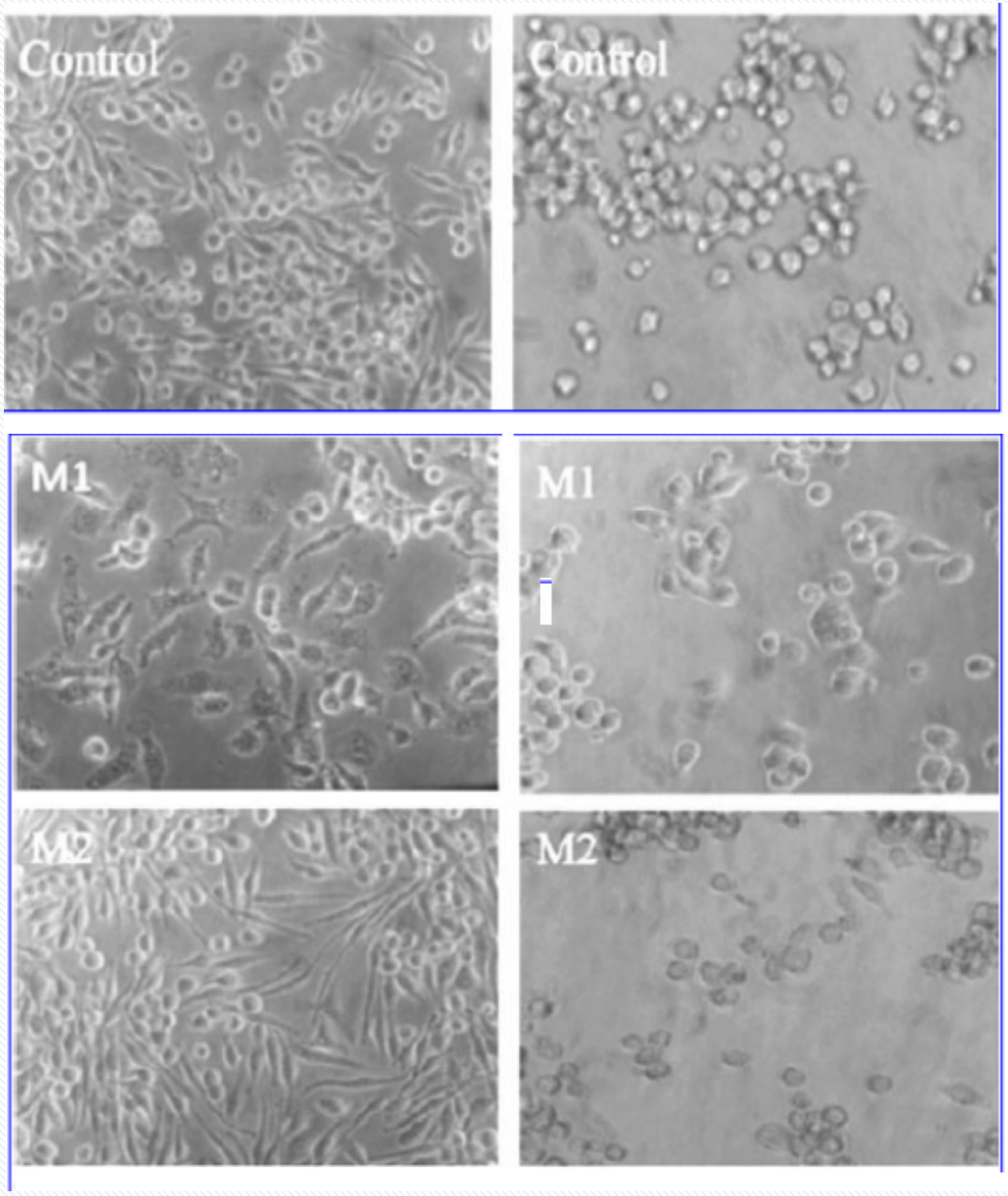


M1 RAW 264.7

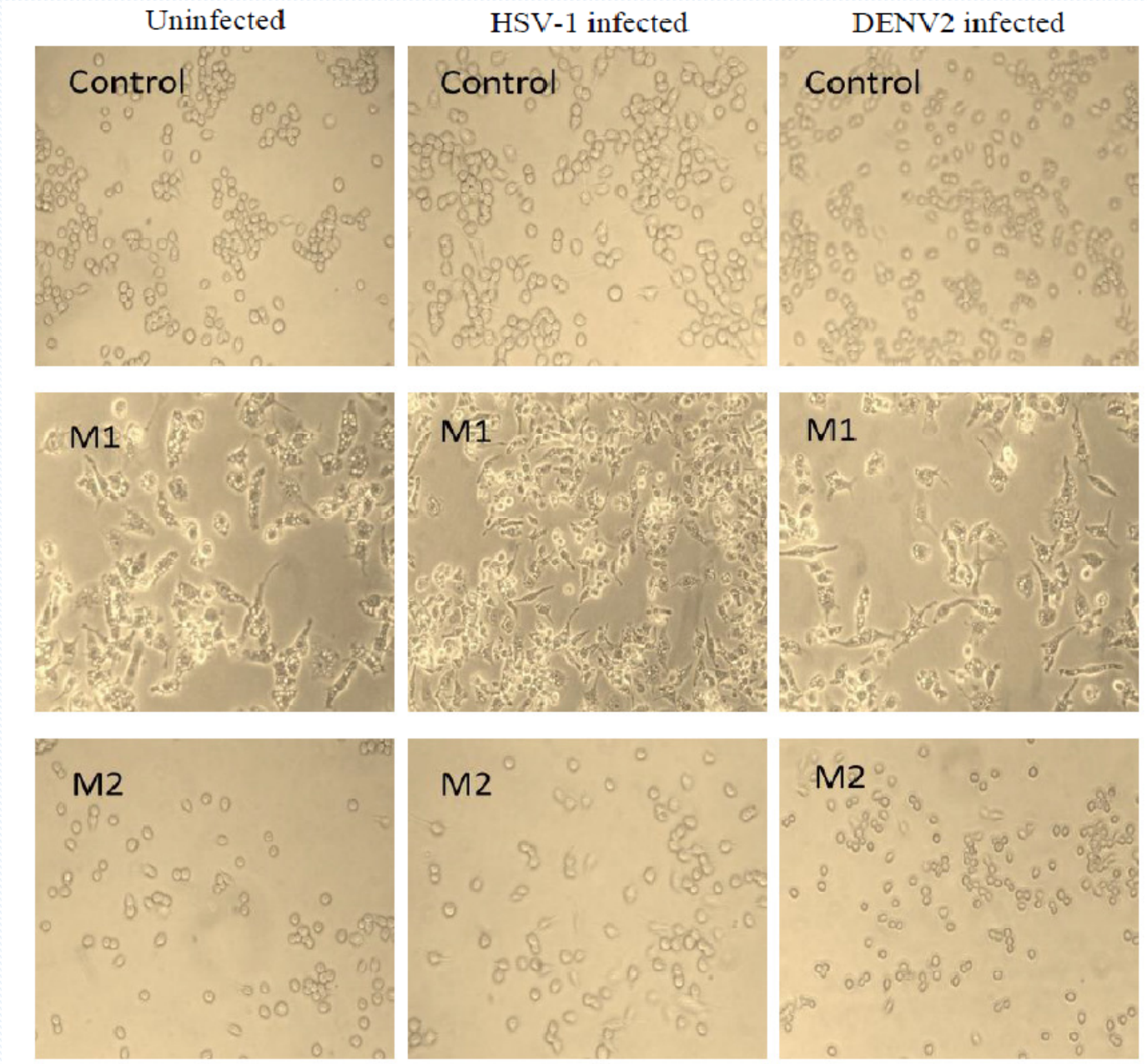
Vacuolated M1 Macrophages

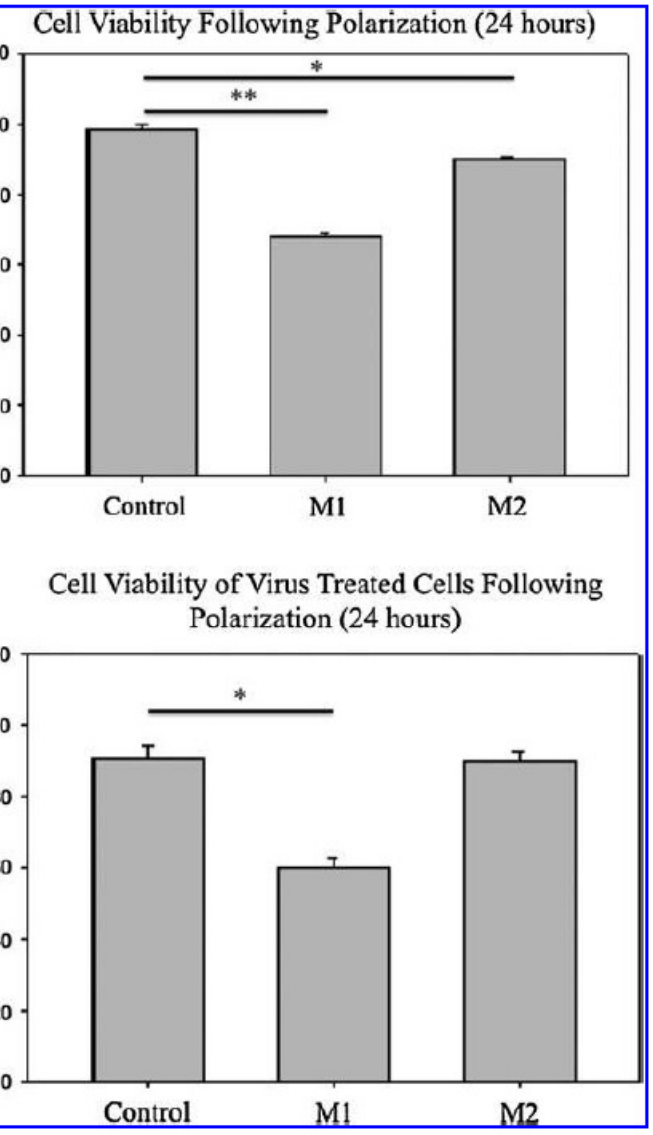
J774A.1 Macrophages

Uninfected HSV-1-infected

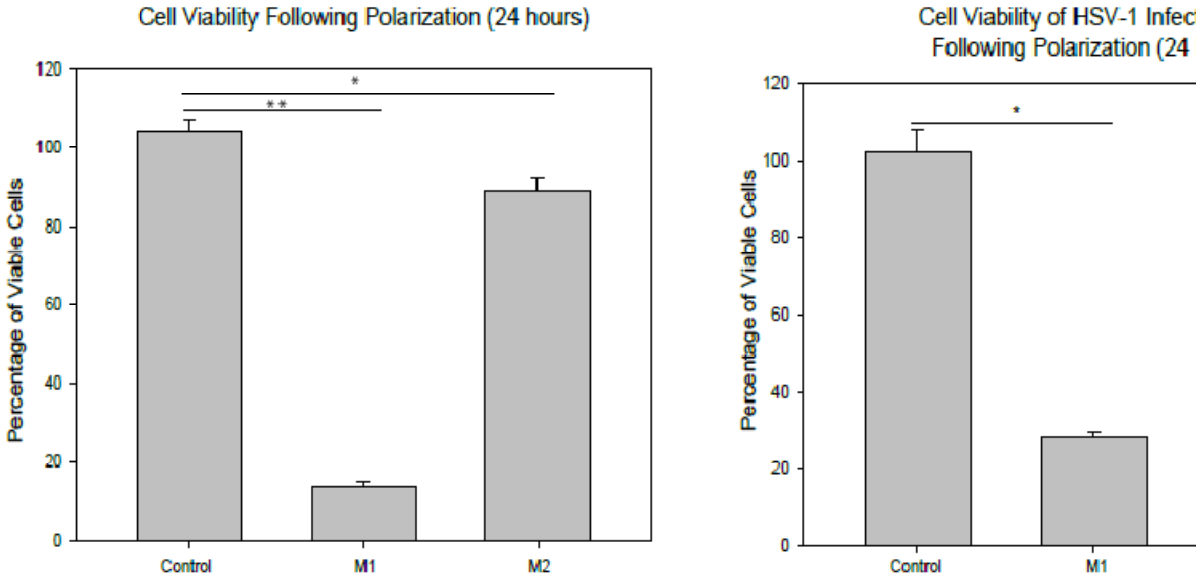


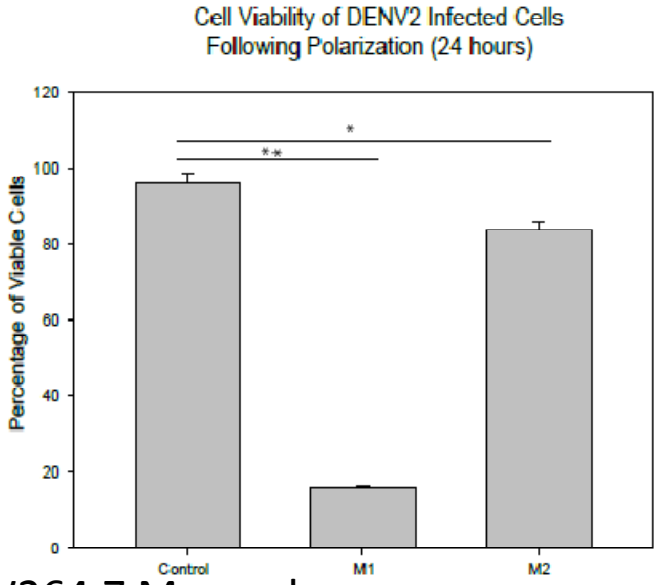
RAW 264.7 Macrophages





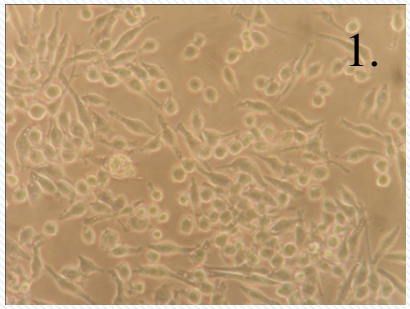
774A.1 Macrophages



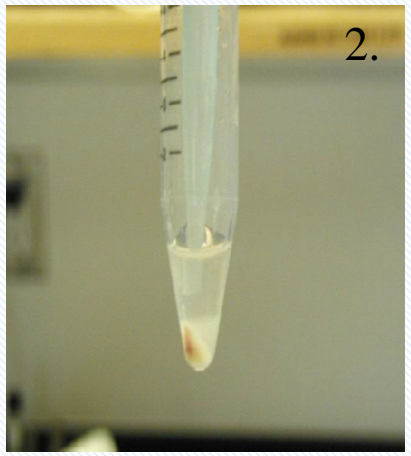


RAW264.7 Macrophages

Virus Treatment of Macrophages

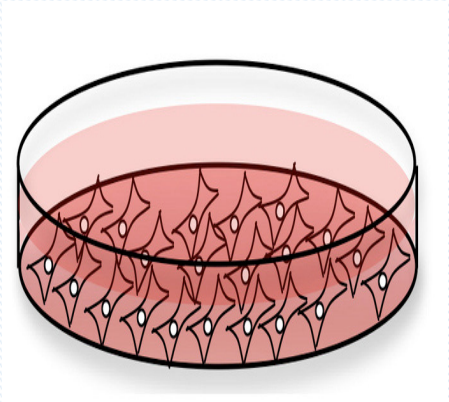


Release cells/centrifuge



Count

Add virus (0.1 MOI)/cytokines



In order to accurately
cell number and calcu
accurately:

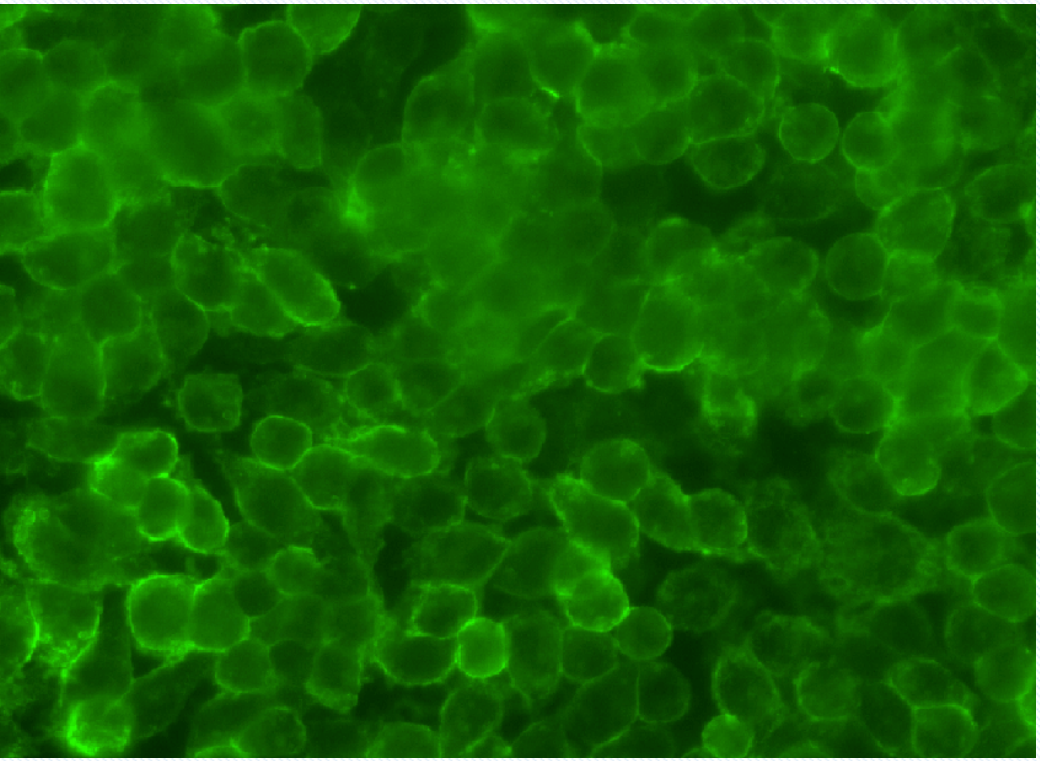
- Cells were released
culture plate using
enzymatic dissocia
reagent

- Cell count was take
TPP PCV cell coun
making it possible
calculate MOI accu

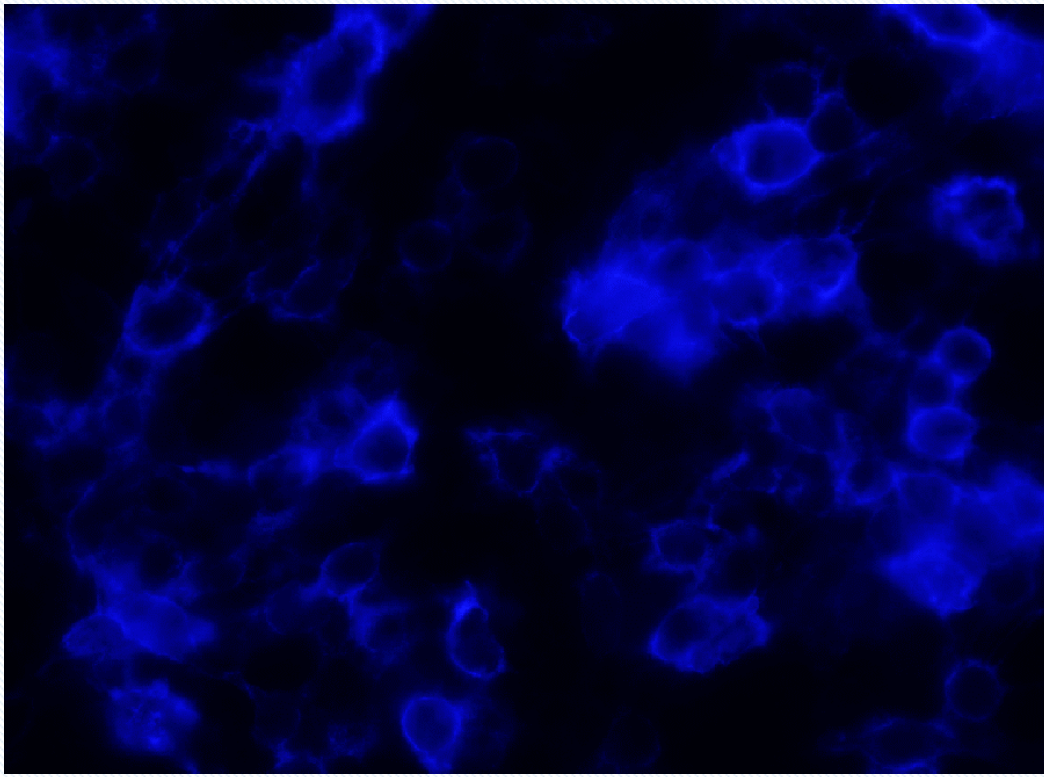
Monocyte/Macrophage Markers	Scavenger Receptors	Membrane Glycoprotein
CD14- LPS receptors	CD206 macrophage mannose receptor (MMR)	CD200R- expressed mainly on monocytes and neutrophils. Interaction between CD200R and CD200 limit and suppress macrophage-induced inflammatory damage.
CD80 (B7.1) co-receptor on antigen –presenting cells (APCs)	CD163- hemoglobin-haptoglobin receptor; expressed on both monocytes and macrophages	
CD86 (B7.2) co-receptor on APSs		

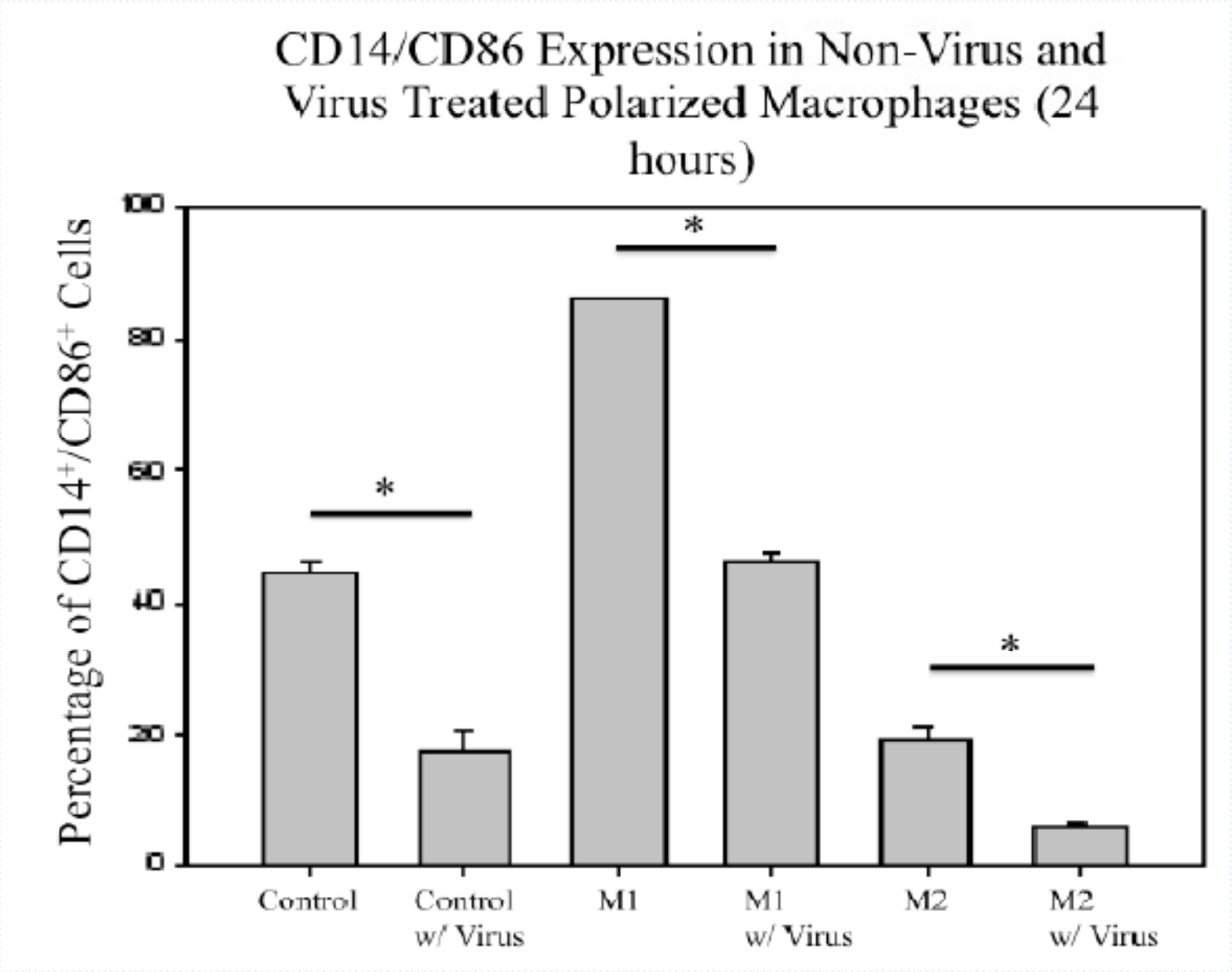
774.1 macrophages polarized to M1 phenotype

tained with FITC-labeled anti- mouse CD14



Stained with brilliant violet 421 - labeled anti-mouse CD86





J774a.1 Murine Macrophages

guin and colleagues recently used monocytes purified from the buffy ats of human peripheral blood cells to characterize phenotypic and nomic markers.

generated macrophages from these primary human cells by treatment with M-CS

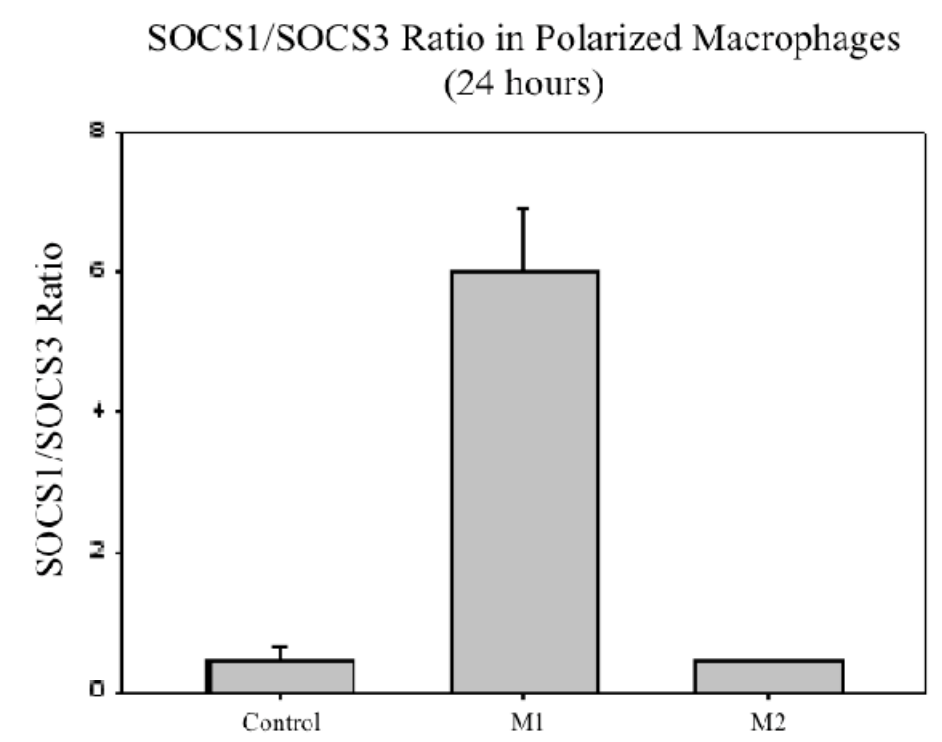
polarized them using the same inducers as used in the present study, LPS and IFN- to induce M1 phenotype and IL-4 to induce the M2 phenotype

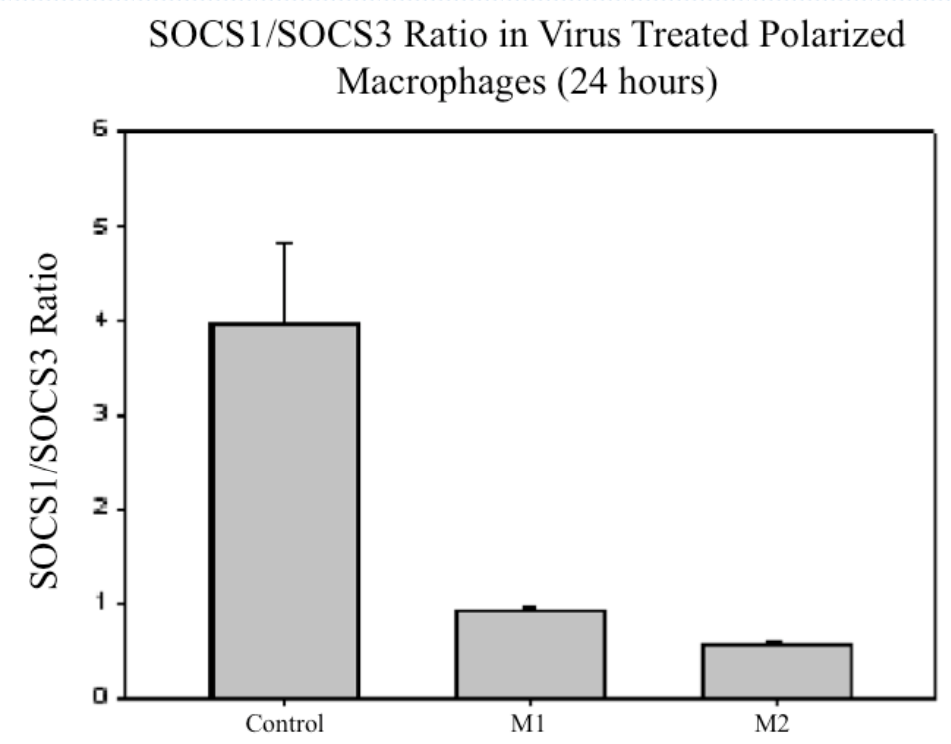
the cell membrane marker unique to M1 cells was CD80 (B7.1)

CD200R expression was unique to the M2 polarized human macrophages

As did we using M1 and M2 polarized murine macrophage cell lines (data not shown), they found that the mannose receptor CD 206 did not distinguish betwee M1 and M2 phenotypes of human macrophages.

tometry summary of SOCS1 and SOCS3 expression by uninfected and infected J774A.1 macroph ulations

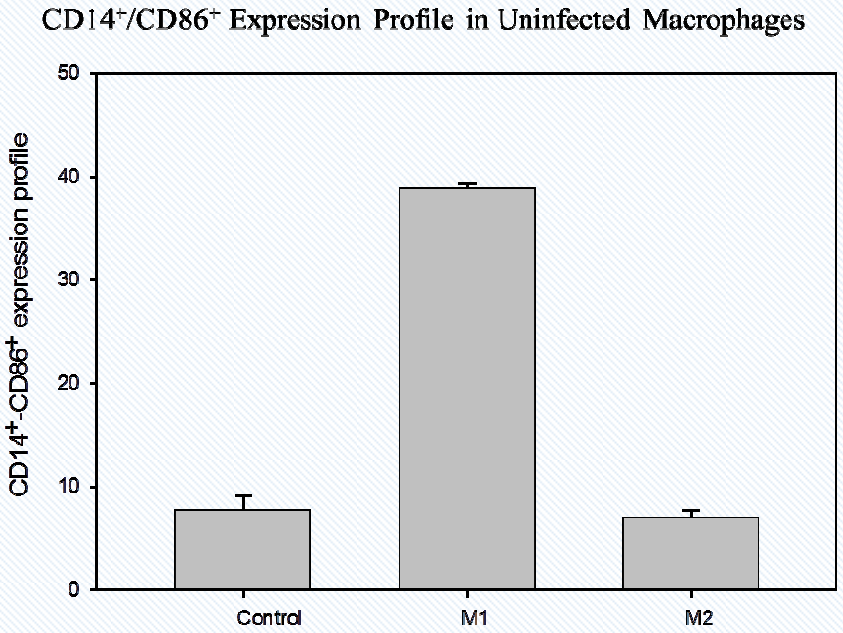


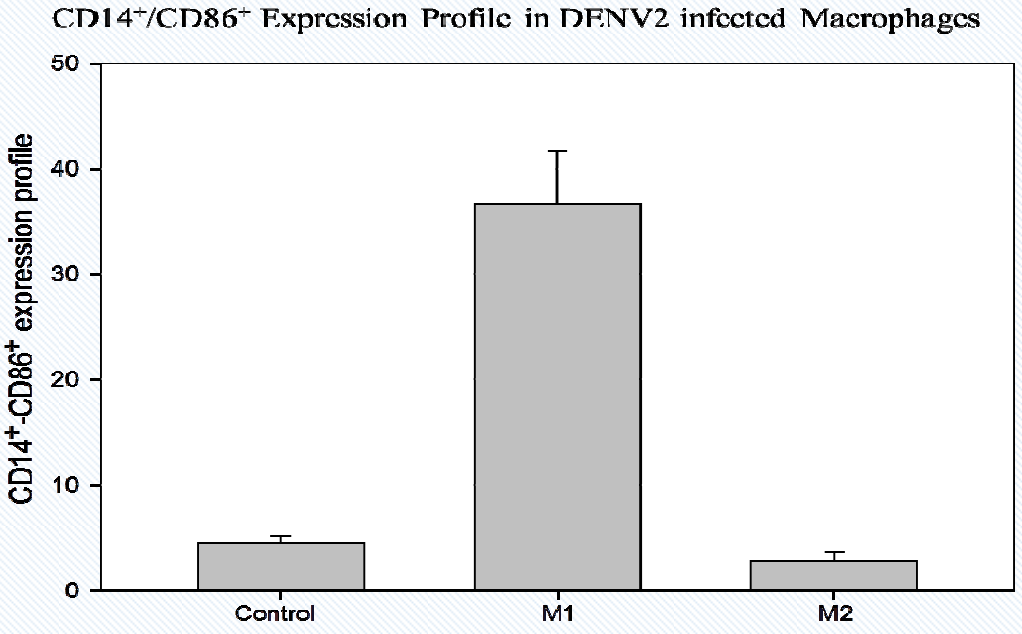


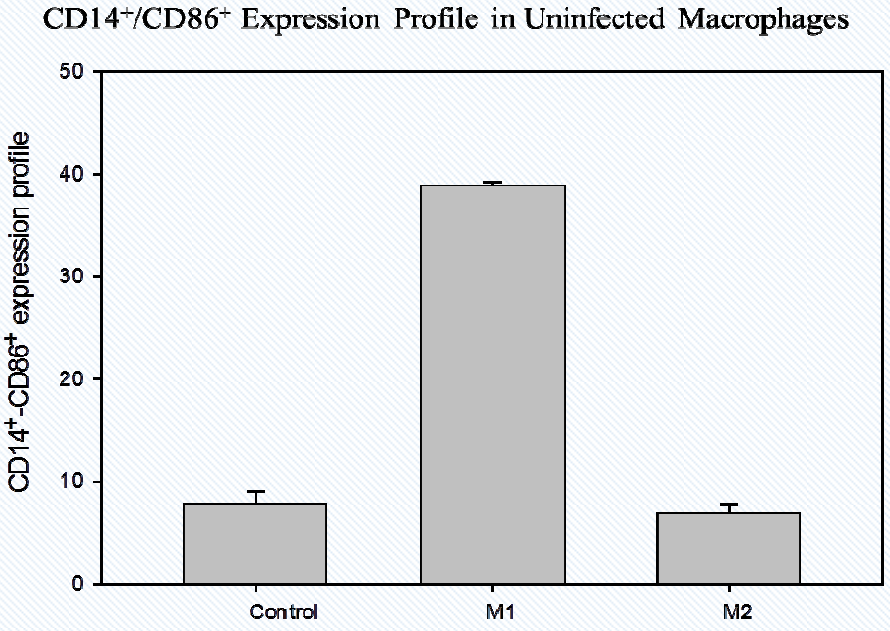
eft Panel. Note Uninfected cells at 24 h after polarization. M1 cells expressed higher levels of SOCS1 than OCS3 with a SOC1/SOCS3 ratio of 7:1.

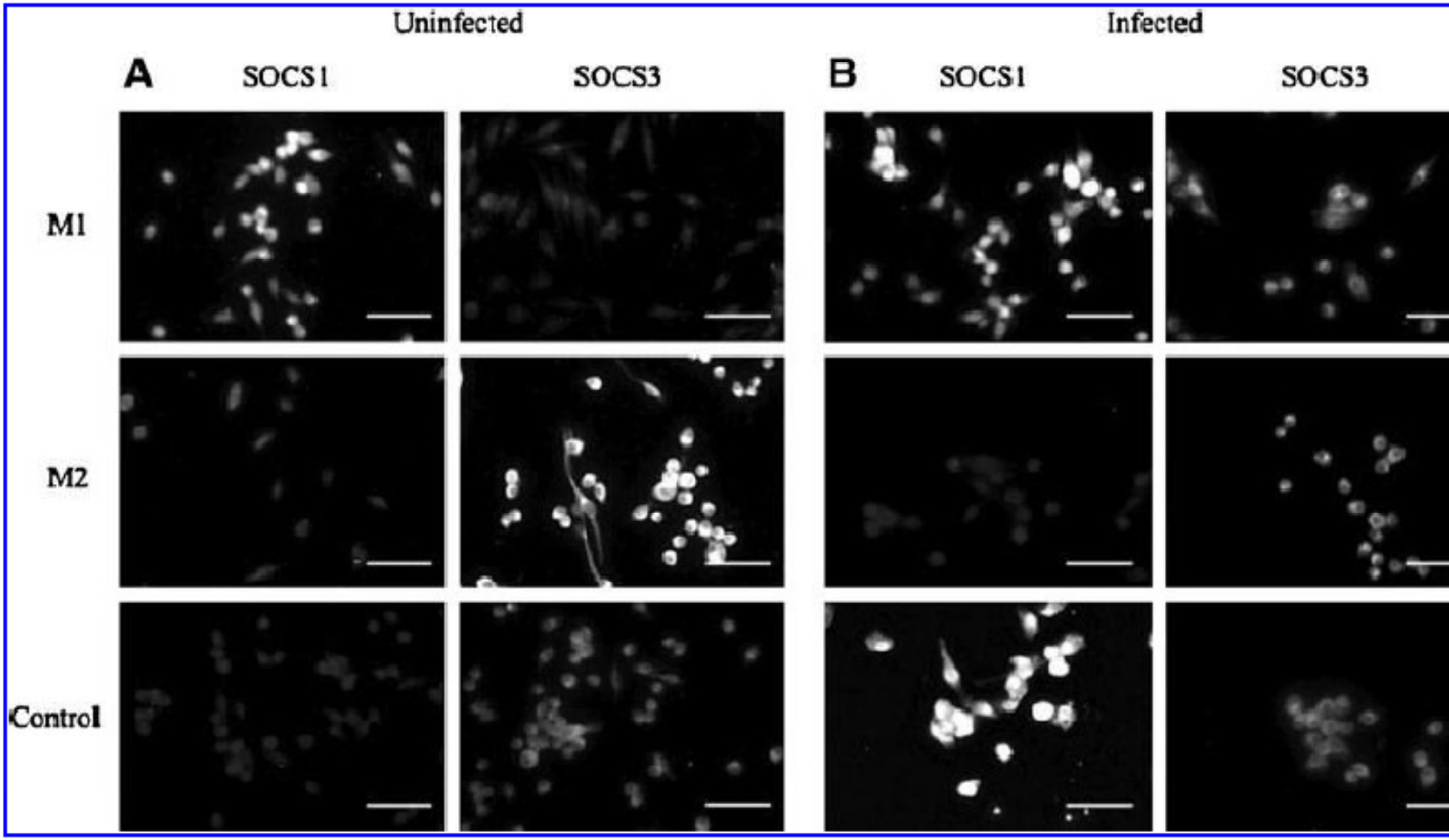
ight Panel. Virus-infected M1 cells expressed a SOCS1/SOCS3 ratio of 1:1 while M2-infected cells exhibited a OCS1/SOCS3 ratio of 1:2

D14/CD86
XPRESSION IN RAW
64.7 Murine
lacrophages








Uninfected
Infected
A
SOCS1
SOCS3
B
SOCS1
SOCS3
M1
M2
Control

os determined by Flow cytometry (J774a.1) and Western Blot (RAW 26

	J774.A	RAW 264.7
	Ratio	Ratio
M0	1:2	1:2
M0-HSV-1	1:2	1:2
M1*	7:1	1:3
M1-HSV-1	1:1	1:1
M2	1:2	1:2
M2-HSV-1	1:2	1:2

* Difference because of cell line or detection method,. Western Blot detects denatured antigenic fragments ; Flow cytometry detects native protein conformation. SOCS1:SOCS3 ratios in all DENV2-infected cells was 1:1.

Summation of Observations

- HSV-1 infection led to morphological differences in all 3 experimental groups
- HSV-1 infection decreased CD14/CD86 expression in all 3 experimental groups
- M1 macrophages did not show an up regulation of SOCS1 following virus challenge, however, SOCS3 levels were increased
- HSV-1-infected unpolarized (M0) J774A.1 cells exhibited significant increases in expression levels of native SOCS1

TABLE 1. HSV-1 TITERS IN HSV-INFECTED UNPOLARIZED AND POLARIZED MACROPHAGE CELL LINES

Cell line/treatment	*pfu/mL*	*Fold decrease from M0*
J774A.1		
M0	10.2×10^2	—
M1	2.5×10^2	4
M2	6.3×10^2	1.4[a]
RAW264.7		
M0	30×10^2	—
M1	10×10^2	3
M2	35×10^2	—

[a]M1 cells demonstrated a 2.5-fold reduction in virus pfu by comparison with infected M2 cells.

HSV, Herpes simplex virus; pfu, plaque forming unit.

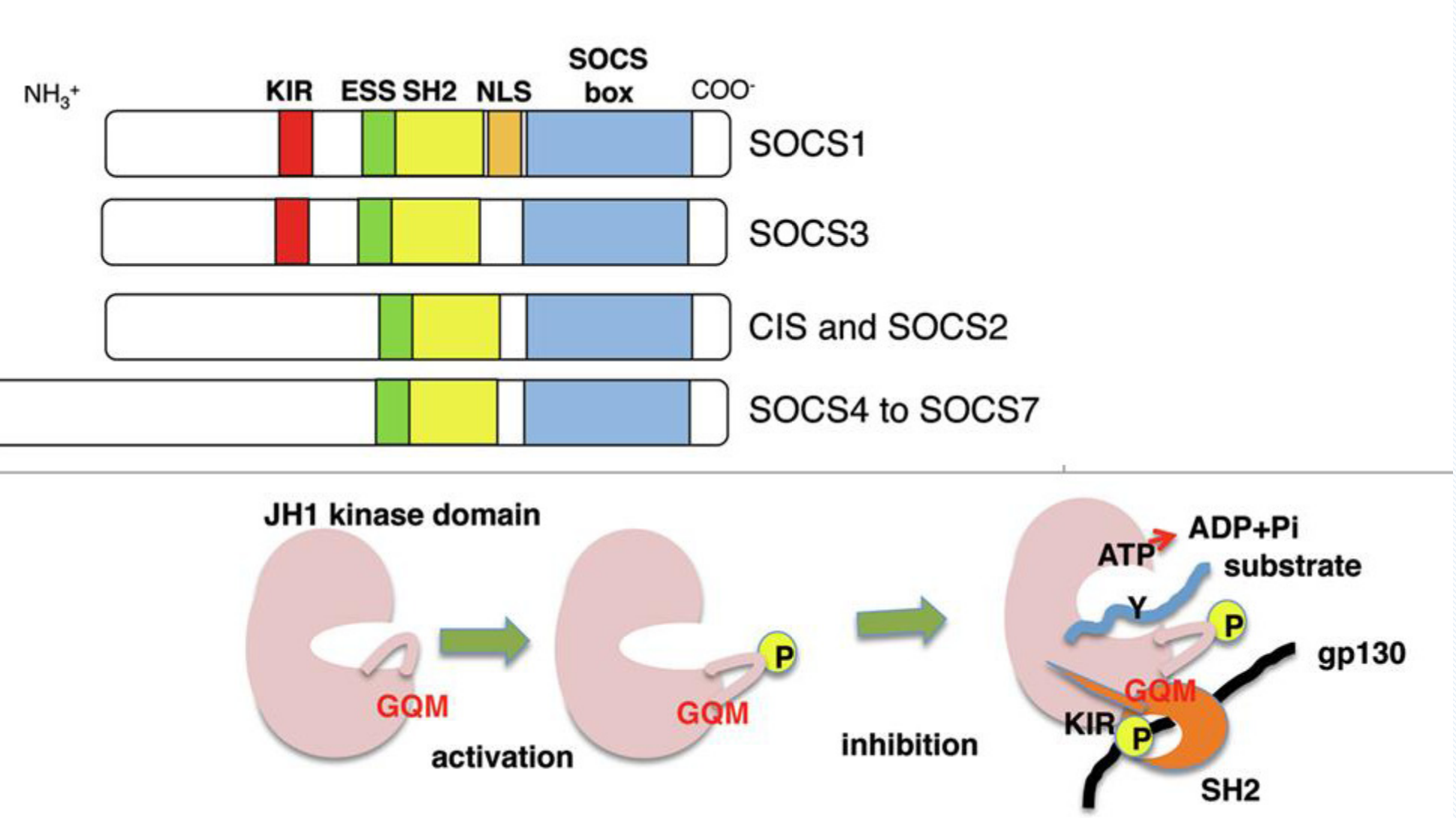
t 24 h after infection, M0 control and M2 cells showed greater virus yield than did the M1 ells, presumably reflecting the loss of viable M1 cells.

es up regulation of SOCS3 expression in HSV-1-infected M1 macrophages over that
n in uninfected M1 cells reflect the effects of M1 polarization or suggest the cell's
empt to counteract effects of proinflammatory molecules?

asimi and colleagues showed that different domains of SOCS3 protein are used to
diate interleukin-10 (IL-10) inhibition of TNF-α and nitric oxide production by this
me macrophage cell line (Qasimi and others 2006). In this same macrophage cell
e (J774A.1) , Il-10 was responsible for the anti-inflammatory response to *Borrelia*
rgdorferi (Dennis and others 2006).

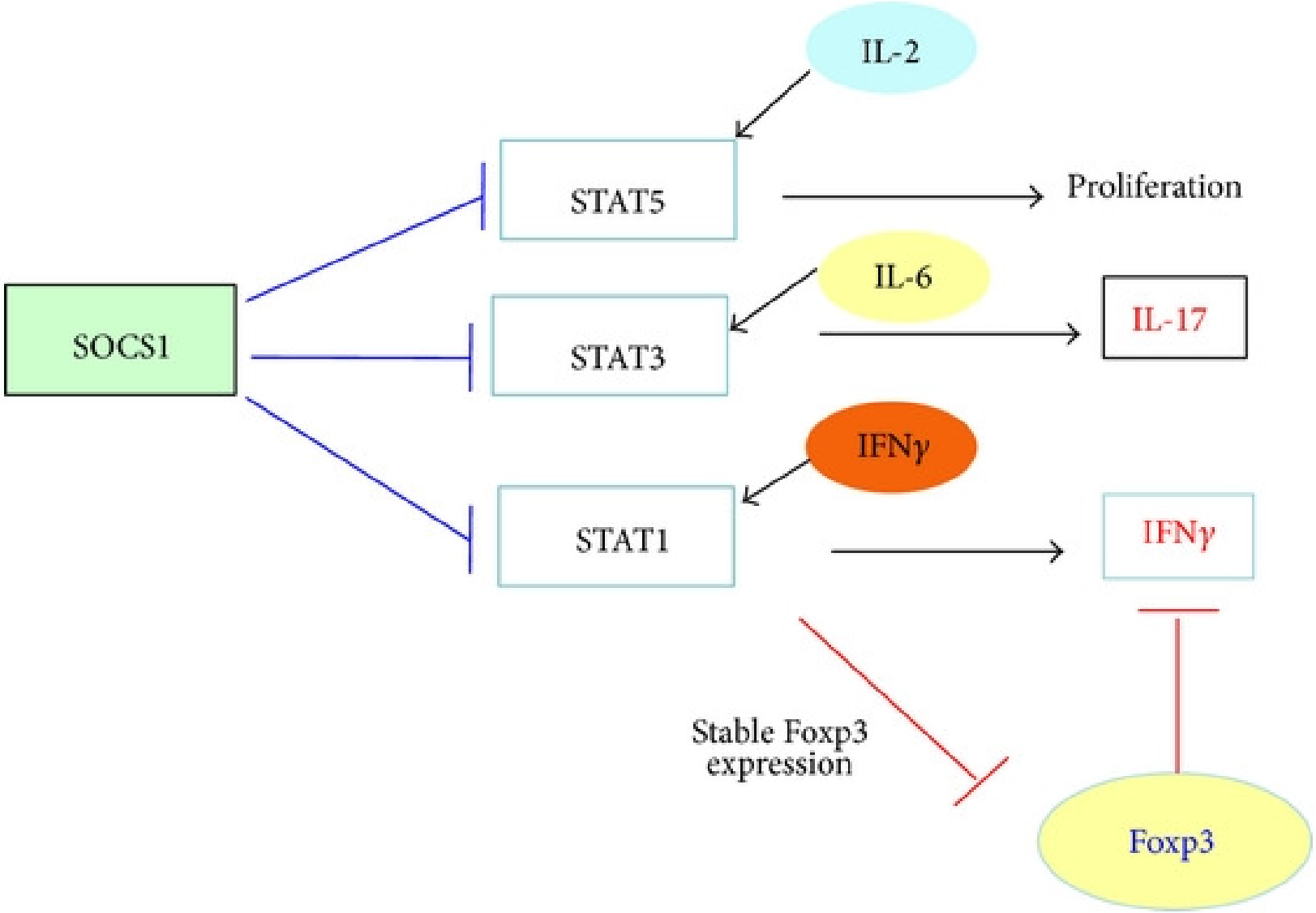
CS1/SOCS3 expression levels appeared relatively unchanged in virus-infected M2
crophages when compared to their uninfected counterparts, suggesting
croenvironment signals such as IL-4 play a greater role in SOCS expression levels
an does HSV-1 infection.

hen hypothesized that the HSV-1-infected J774A.1 M1 macrophages were
apting to counteract the effects of inflammatory molecules induced by
ization.

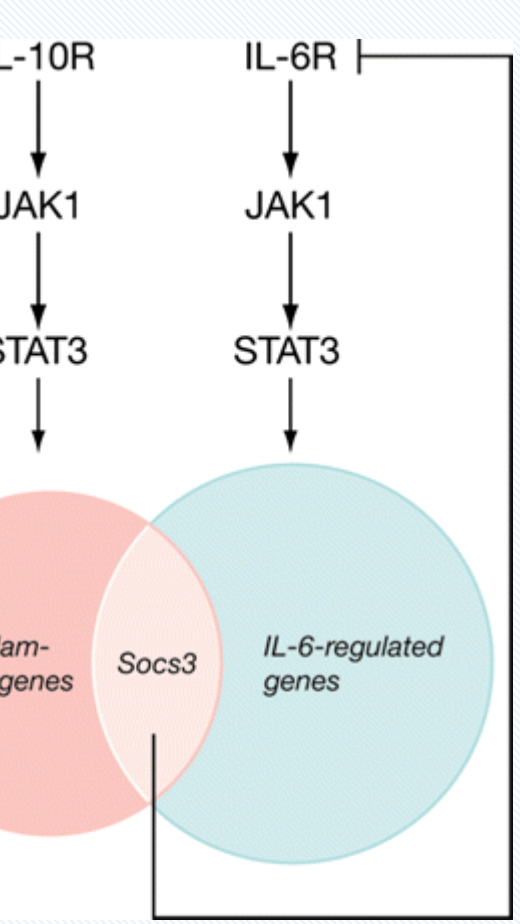


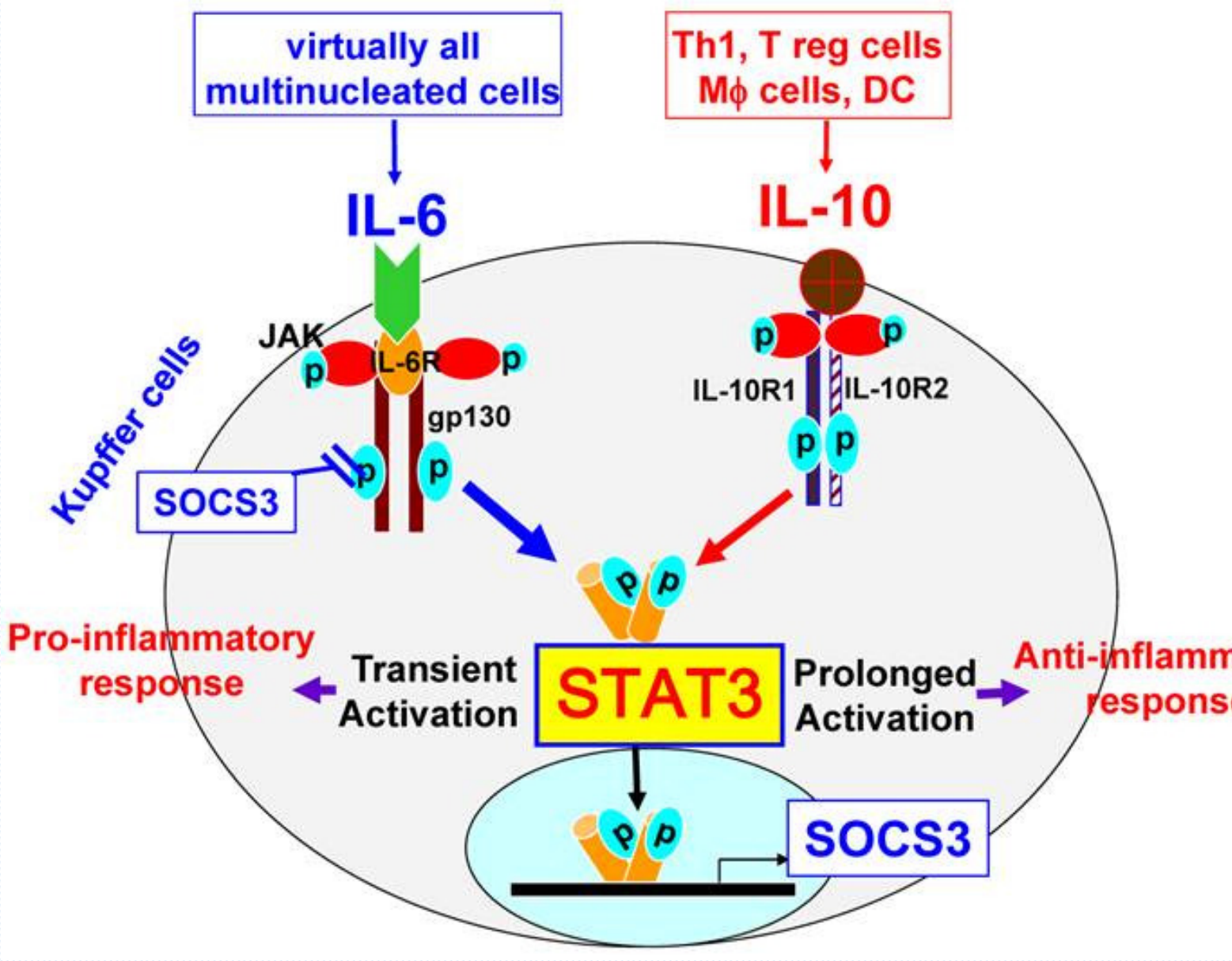
Structural domains of SOCS molecules

Note that only SOCS1 contains a nuclear localization sequence (NLS)



Overview of SOCS1 inhibition of cytokine-induced STATs

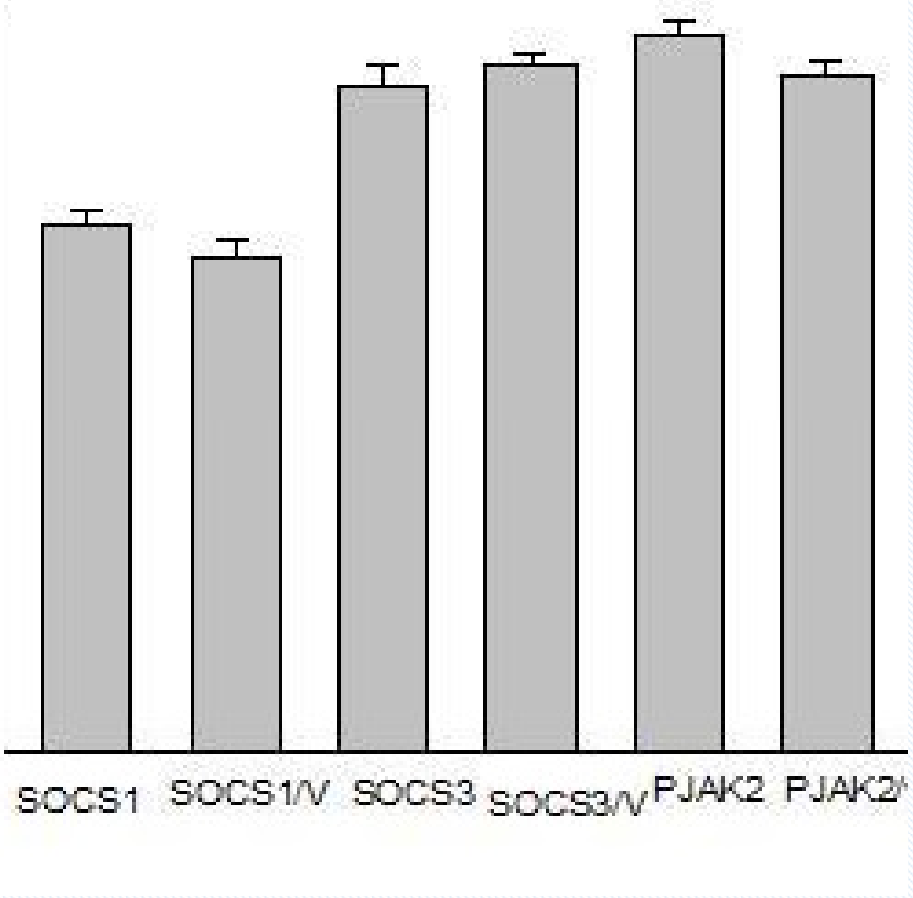




Pro-inflammatory and anti-inflammatory effects of SOCS3

- LPS (100 ng/ml)
- IFN-γ (20 ng/ml)
- 0.1 MOI HSV-1
- SOCS3 peptide or SOCS1 Inhibitor

Incubate 24 hours



p<0.001 when the SOCS
were compared with th
and pJAK2 groups

s of SOCS1 and SOCS3 peptide mimetics and SOCS1 inhibitor (pJAK2) on polarized
.1 macrophages.

Jo et al. (2005) used a recombinant cell-penetrating form of SOCS3 (CP-SCS3) 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 .

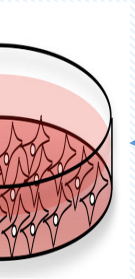
Within 2 hours after injection, CP-SOCS3 was distributed In multiple organs and persisted for at least 8 hours

The membrane-translocating motif (MTM) was composed of 12 amino acids from a hydrophobic signal sequence form fibroblast growth factor 4. The MTM was attached to either the N- terminal or C-terminal of SOCS3. Only these forms were capable of penetrating RAW cells.

Based on these observations, we tested whether the SOCS3 peptide mimetic could modify the cytotoxicity of the M1 polarization treatment or virus infection.

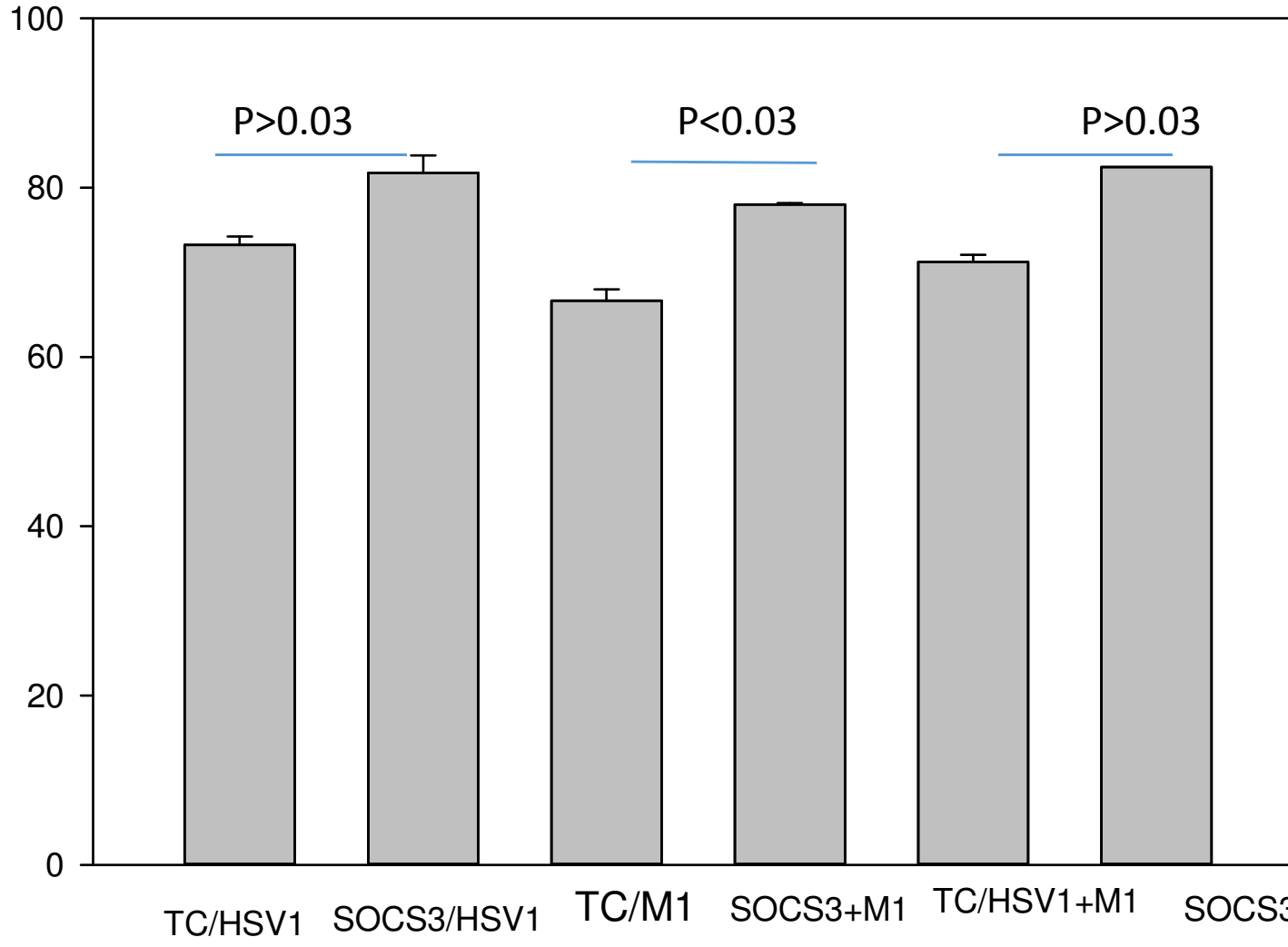
The peptide mimetics in this present study were provided by Dr. H.M. Johnson and his colleagues , University of Florida at Gainesville. These peptides contain an addition of a lipophilic group (palmitoyl-lysine) to the N terminus of the synthetic peptide which provides them with the ability to penetrate cells.

CS3 Peptide
metic protects
crophages (RAW
.7) from the lytic
ect of HSV-1 and
m the lytic effect of
polarization



- SOC3 peptide (35µM/ml) or TC medium for 30 minutes prior to
- LPS (100 ng/ml)
- IFN-γ (20 ng/ml)
- 0.1 MOI HSV-1

Incubate 24 hours



Cell Viabilities of RAW 264.7 macrophages 24 hours after M1 polariz

CONCLUSIONS

S0CS3 peptide mimetic and the S0CS1 inhibitor (pJAK2) increased the viability of polarized M1 cells over SOCS1-treated M1 J 774A.1 macrophages similar to the observations in comparable cell groups infected with HSV-1 ($p<0.001$)

Prediction: The anti-inflammatory effect in these cells will be characterized by increased levels of IL-10

SOCS1 peptide mimetic decreases the viability of polarized M1 cells and HSV-1-infected M1 J774A.1 macrophages ($p<0.001$)

Prediction: The inflammatory effect in these cells will be characterized by increased levels of TNF-α.

SOCS3 Peptide Mimetic protects macrophages (RAW 264.7) from the lytic effect of HSV-1 and from the lytic effect of M1 polarization

These characterization are in progress at present.

Significance

- Benefits of SOCS3 Peptide Mimetic

  Neuro inflammation- already shown in microglial cells by Benveniste's group (Qin et al, 2012).

  Anti-inflammatory effects in inflammatory diseases including viral diseases such as Dengue fever and autoimmune tissue destruction.

- Benefits of SOCS1 Peptide Mimetic

  Convert the M2-type macrophage in solid tumors to an inflammatory M1 phenotype

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