● PERSPECTIVE

The suppressor of cytokine signalling 2 (SOCS2), traumatic brain injury and microglial/macrophage regulation

Traumatic brain injury (TBI) results in a range of neuroinflammatory events that vary depending on the type and extent of injury. Central to this is the activation of tissue resident microglia and infiltration of peripheral macrophages, which phagocytose debris and/or secrete a range of cytokines, chemokines and other factors which modify the injured environment to promote or inhibit repair (Schwartz et al., 2013). The reactive macrophages/microglia are broadly divided into two categories, the classical pro-inflammatory (M1) and alternative anti-inflammatory (M2) phenotypes. Much research has been undertaken to try promote the M2 phenotype to aid in neural repair as the balance of pro- and anti-inflammatory phenotypes dictates whether or not tissue repair can occur. However, it is now clear that there is not a simple M1/M2 dichotomy but rather a broad spectrum of phenotypes that are more or less pro- or anti-inflammatory (Kim et al., 2016). *In vitro*, lipopolysaccharide (LPS) or interferon gamma (IFNγ) stimulation promotes the M1 phenotype and production of pro-inflammatory cytokines, while interleukin (IL)4/IL13 promote cells with the anti-inflammatory M2 phenotype, which promotes tissue repair and resolves inflammation. However, this relatively simple characterization does not appear to hold up *in vivo*, for example following TBI (Kim et al., 2016).

Recently we showed that suppressor of cytokine signalling 2 (SOCS2) may be a player in macrophage/microglial response following TBI (Basrai et al., 2016). SOCS2 has been shown to act as an important regulator of inflammatory responses in the periphery and roles for it in directing macrophage polarization towards an anti-inflammatory phenotype have been suggested (Machado et al., 2006; Esper et al., 2012; Zhou et al., 2014; Brant et al., 2016).

We compared the effect of TBI in SOCS2 over-expressing transgenic (SOCS2Tg) mice to wild-type (WT) littermates (Basrai et al., 2016). Overexpression of SOCS2 promoted recovery following moderately severe TBI, with SOCS2Tg mice showing functional improvement on a ladder test, with a smaller lesion volume at 7 days post injury. Following mild TBI, the number of proliferative $CD11b^+$ macrophage/microglial cells that were present in the injured cortex of SOCS2-overexpressing mice at 35 days post-injury was almost double that observed in WT mice, with no differences in the un-injured contralateral cortex. However, this effect was not significant at 35 days following more severe TBI, but with a modest effect at 7 days post-TBI, suggesting a role of injury severity and timing in the extent of microglial/macrophage activation that is regulated by SOCS2 (Basrai et al., 2016). Given that the SOCS2-over-expressing mice showed functional improvement compared to WT mice following moderately severe TBI, we did not expect a difference in total activated microglial/ macrophage cell numbers but rather thought it may be due to altered M1/M2 polarisation regulated by SOCS2. Immunohistochemistry for M1 and M2-like macrophages/microglia 7 days post moderate injury suggested an altered activated macrophage/microglial response in SOCS2Tg compared to WT mice. Specifically, the greater area covered by $CD206^+$ cells in SOCS2Tg perilesional cortex suggested the presence of more M2-like macrophages/microglia than in the WT perilesional cortex (Basrai et al., 2016). Therefore, the expression of M1 and M2 macrophage/microglia associated genes was further analyzed by q-PCR to further elucidate changes in the balance of M1 and M2 microglia in SOCS2Tg and WT mice 7 days post moderate injury. No differences in gene expression were present between SOCS2 naïve and WT naïve mice. For injured SOCS2Tg and WT mice, gene expression was expressed as fold change after injury compared to naïve mice. All M1 and M2 associated genes showed an up-regulation of RNA expression after injury verses naïve control in SOCS2Tg and WT mice but no differences were seen in M1 and M2 marker expression between SOCS2Tg and WT mice (**Figure 1**).

These findings correlate with a recent single cell RNA sequencing analysis of macrophage activation states following TBI (Kim et al., 2016). It was found that the macrophages expressed mixed polarisation signatures, with different individual cells apparently randomly co-expressing different sets of polarisation markers. Approximately 70% of monocytes at 24 hours post-TBI expressed SOCS2 but co-expression of other key polarisation markers, such as pro-inflammatory IL1b and tumor necrosis factor or anti-inflammatory Arg1 or Chi3L3 was not different between SOCS2 expressing or non-expressing cells. Therefore, while SOCS2 is involved in regulating the immune response, it does not appear to do so following TBI by regulating pro- or anti-inflammatory monocyte phenotype, at least in terms of level of expression of M1- or M2-associated genes. How then, may SOCS2 be acting in this regard?

Following mild TBI there was a significantly increased number of proliferative (EdU^+) $CD11b^+$ macrophages/microglia in SOCS2Tg mice compared to WT, while following moderately severe TBI, WT $EdU^+/CD11b^+$ cell numbers caught up to those in SOCS2Tg mice (Basrai et al., 2016). This may indicate that SOCS2Tg mice have microglia/macrophages that are hypersensitive or better primed to respond to immune challenge compared to WT mice. This may have resulted in a higher baseline of proliferation or enhanced migration, allowing a more robust response to TBI. Further studies are required to elucidate the mechanisms involved. These may include dysregulated responsiveness to a range of inflammatory modulators, such as IFNγ or Toll like receptor (TLR)/nuclear factor (NF)-κB signalling pathways. SOCS2KO mice fed a high fat diet have an increased expression of pro-inflammatory mediators such as IFN-γ in their livers and adipose tissue (Zadjali et al., 2012), while analysis of bone marrow derived macrophages from SOCS2KO mice demonstrated an increased phagocytic ability, secreted more pro-inflammatory cytokines and had decreased levels of the NF-κB inhibitor IκBα (Zadjali et al., 2012). Indeed LPS induced TLR/NF-κB signalling has been shown to induce SOCS2 in a STAT3 and STAT5 dependent manner in human dendritic cells (Hu et al., 2012). A more detailed analysis of the timing of SOCS2's effects will likely also shed more information on potential mechanisms of action. In particular, studies at time points earlier than 7 days may be most informative, given that the microglial/macrophage response commences within hours of injury.

Clearly, the macrophage/microglial response to TBI is apparently more complex than previously thought. Standard M1/M2 polarisation rules derived *in vitro* do not appear to apply, with individual cells expressing multiple different polarisation markers along the M1/M2 spectrum. It is also apparent that the response is regulated by genes such as SOCS2 that, while on first glance, would seem likely to regulate polarization by promoting an anti-inflammatory phenotype, instead appear to do so by mechanisms other than, or in addition to, regulating expression of specific polarisation genes. This may include regulation of responsiveness to activation of different signal transduction pathways that regulate proliferation, migration or possibly cell

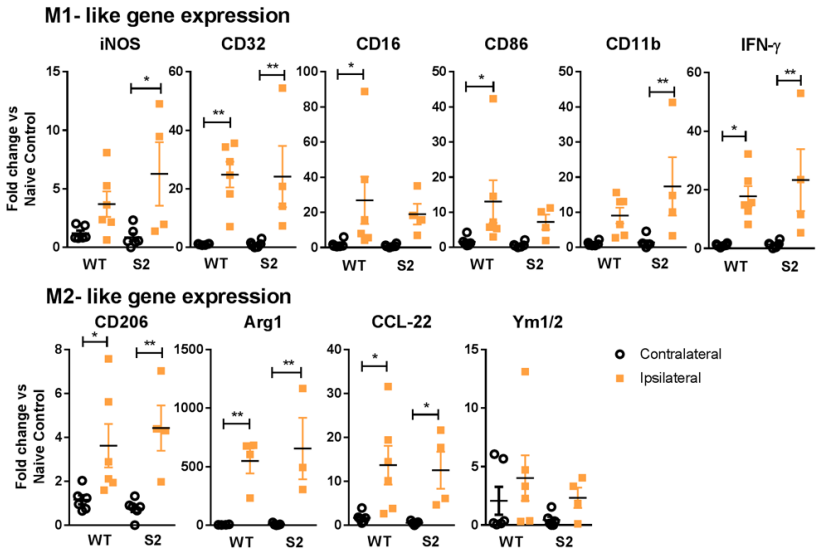

Figure 1 M1 and M2-like macrophage/microglia gene expression was similar in the cortex of SOCS2Tg and wild-type (WT) mice 7 days post moderately-severe traumatic brain injury (TBI).

Fresh cortical tissue was obtained from SOCS2Tg and WT mice 7 days post moderate injury (Basrai et al., 2016) as well as naïve mice and analyzed by q-PCR for expression of various M1 and M2 macrophage/microglia-like genes, with methods essentially as previously described (Turbic et al., 2011). Gene expression was analyzed as a fold change after TBI versus naïve control and the ipsilateral and contralateral cortex of injured mice compared. M1-like genes iNOS, CD32, CD16, CD86, CD11b, IFN-γ and M2-like genes CD206, Arg1, CCL-22 and Ym1/2 all showed an increase in expression after injury relative to naïve. Results are shown as the mean ± SEM; $n = 3$–6 mice/group; $^{*}P < 0.05$, $^{**}P < 0.01$ (one-way analysis of variance with Bonferroni *post hoc*; A–J). The primers for each of the genes are as follows (5′–3′; F – forward, R – reverse): iNOS, F: CAA GCA CCT TGG AAG AGG AG/R: AAG GCC AAA CAC AGC ATA CC; CD32, F: AAT CCT GCC GTT CCT ACT GAT C/R: GTG TCA CCG TGT CTT CCT TGA G; CD16, F: TTT GGA CAC CCA GAT GTT TCA/R: GTC TTC CTT GAG CAC CTG GAT C; CD86, F: GAC CGT TGT GTG TGT TCT GG/R: GAT GAG CAG CAT CAC AAG GA; CD11b, F: CCA AGA CGA TCT CAG CAT CA/R: TTC TGG CTT GCT GAA TCC TT; IFN-γ, F: ACA CTG CAT CTT GGC TTT GC/R: CGA CTC CTT TTC CGC TTC CT; CD206, F: CAA GGA AGG TTG GCA TTT GT/R: CCT TTC AGT CCT TTG CAA GC; Arg1, F: TCA CCT GAG CTT TGA TGT CG/R: CTG AAA GGA GCC CTG TCT TG; CCL-22, F: CTG ATG CAG GTC CCT ATG GT/R: GCA GGA TTT TGA GGT CCA GA; Ym1/2, F: CAG GGT AAT GAG TGG GTT GG R: CAC GGC ACC TCC TAA ATT GT and GAPDH control, F: TCC CAG AGC TGA ACG GGA AG/R: TCA GTG GGC CCT CAG ATG C. iNOS: Inducible nitric oxide synthase; IFN-γ: interferon γ: CCL-22: C-C motif chemokine ligand 22.

maturation. Given the role of SOCS proteins in regulating activation of signal transduction pathways downstream of multiple different cytokines, chemokines and other growth factors, more work is needed to elucidate the mechanisms by which SOCS2 regulates the macrophage/microglial response following TBI to result in enhanced recovery. Identification of such pathways or mechanisms may provide new therapeutic targets which may be exploited to enhance recovery following TBI.

This work is supported by the National Health and Medical Research Council of Australia; Project grant 1045125 and Fellowship (to AMT) 628344 and support from Stem Cells Australia.

Harleen S. Basrai, Ann M. Turnley*

Department of Anatomy and Neuroscience, The University of Melbourne, Melbourne, Australia

***Correspondence to:** Ann M. Turnley, Ph.D., turnley@unimelb.edu.au.*
***Accepted:** 2016-09-01*
***orcid:** 0000-0002-8442-127X (Ann M. Turnley)*
***doi:** 10.4103/1673-5374.191206*
***How to cite this article:** Basrai HS, Turnley AM (2016) The suppressor of cytokine signalling 2 (SOCS2), traumatic brain injury and microglial/macrophage regulation. Neural Regen Res 11(9):1405-1406.*

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