SOCS PROTEINS AND JAK-STAT PATHWAY DYSREGULATION IN SIV-INFECTED SUPPRESSED MACAQUES

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ABSTRACT

BACKGROUND: Suppressor of cytokine signaling (SOCS) is a family of proteins upregulated rapidly in response to stimulation by Toll-like receptors, cytokines, grow factors and hormones that provide a negative feedback to the stimulation that triggered them by inhibiting the JAK-STAT signaling pathway. SOCS proteins, particularly SOCS3, have also been described as having a central role in metabolic syndrome, diabetes and atherosclerosis. *In vivo* data for SOCS levels in HIV-infected patients are very limited.

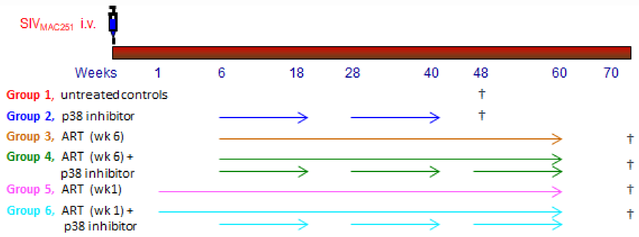
METHODS: Using intracellular staining (ICS) and flow cytometric analyses, we evaluated the expression and the accumulation level, estimated via MFI, of SOCS1, SOCS3, TLRs, IFNs and other JAK-STAT signaling pathway-related proteins in blood and lymph node MNC, harvested at day 0, peak of infection, and week 20, and 60 from SIV-infected Rhesus macaques left untreated, treated with ART or ART+p38MAPK inhibitor. Boolean data analysis permitted the evaluation of co-expression of the above proteins.

RESULTS: In the context of untreated or treated chronic HIV or SIV infection, a persistent but aberrant activation of SOCS proteins and their targets is an important feature of the dysfunctional TLR-IFN-SOCS pathway. The percentage of SOCS+ cells remains higher than at peak viremia after 54-59 weeks of ART despite virus suppression and its expression does not correlate with viral loads. SOCS1 and SOCS3 expression is elevated in virtually all mononuclear cell subpopulations yet the inhibition of their targets JAK and STAT is not complete and markers of innate immunity that should be impacted by SOCS activity remain elevated.

CONCLUSIONS: Persistent SOCS protein expression during suppressed SIV infection supports the existence of additional stimulation that maintains their expression and/or dysregulation of their negative feedback. Incomplete JAK-STAT pathway suppression by SOCS proteins is consistent with residual activation of innate immunity pathways and dysregulation of antiviral immunity. Given the association of their expression with metabolic conditions, SOCS protein chronic activation could be also relevant to the metabolic complications observed in ART patients.

METHODS:

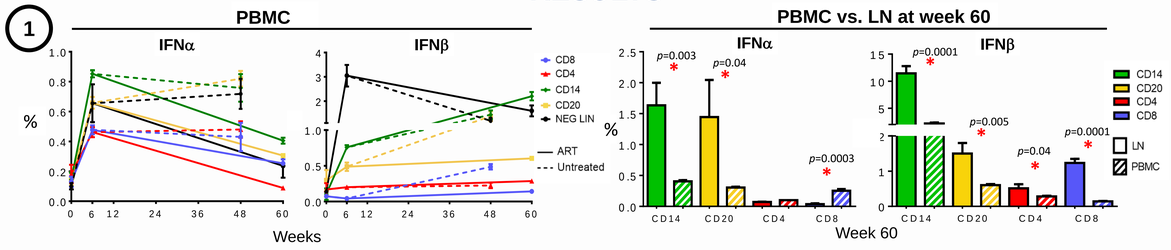
A. SOURCE OF SAMPLES: SIV-INFECTED, ART TREATED RHESUS MACAQUES



B. ANTIBODY PANEL USED FOR THE STUDY IN FLOW CYTOMETRIC ANALYSIS

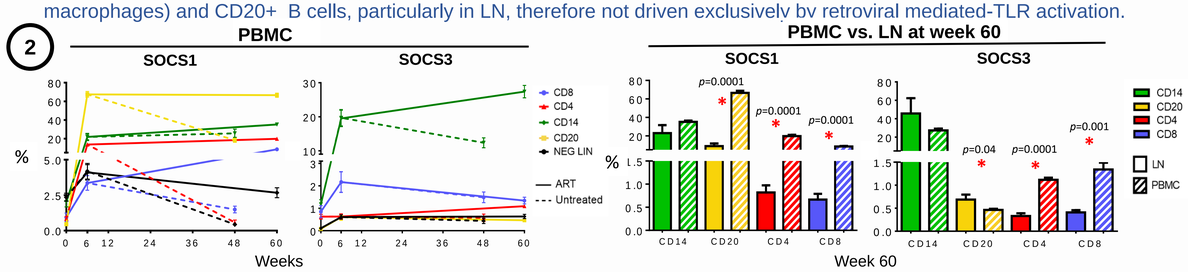
PANEL	APC-C7	PERCP	V500	PE-CY5	BV421	BV605	AF647	AF488	AF594	AF700	DyL350	PE	FITC	PB
1	CD3	CD4	CD8	CD20	CD14	--	SOCS1	--	SOCS3	IFN-α	--	--	IFN-β	USP18
2	CD3	CD4	CD8	CD20	CD14	--	SOCS1	SOCS3	TLR7	TLR8	TLR2	IL-10	--	TLR4
3	CD3	CD4	CD8	CD20	CD14	--	Mx1	--	OAS2	Mx2	--	OAS1	--	JAK1

RESULTS



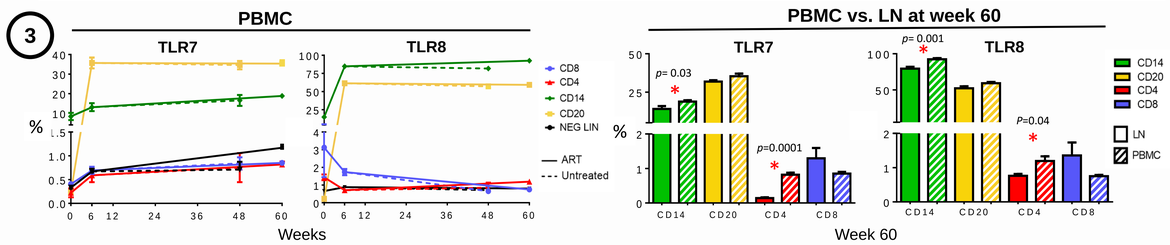
- IFNα percentages decrease substantially in all PBMC populations of ART+ treated animals after initiation of ART treatment ($p<0.01$), paralleling the reduction of viremia, however they did not return to normal in most populations despite viremia suppression.
- IFNβ percentages in PBMC did not significantly changed with ART and were mostly comparable to those of in CD4+, CD14+ and Lin- of untreated animals. Significantly lower percentages were observed in PBMC B cells ($p<0.03$) and CD8+ T-cells of treated animals compared to untreated animals ($p<0.04$).
- Percentages of IFNα and IFNβ were significantly higher in CD14+ and CD20+ LN MNC than in PBMC of treated animals.

After prolonged ART treatment, Type I IFN production is predominantly due to its expression in CD14+ (monocytes and/or macrophages) and CD20+ B cells, particularly in LN, therefore not driven exclusively by retroviral mediated-TLR activation.



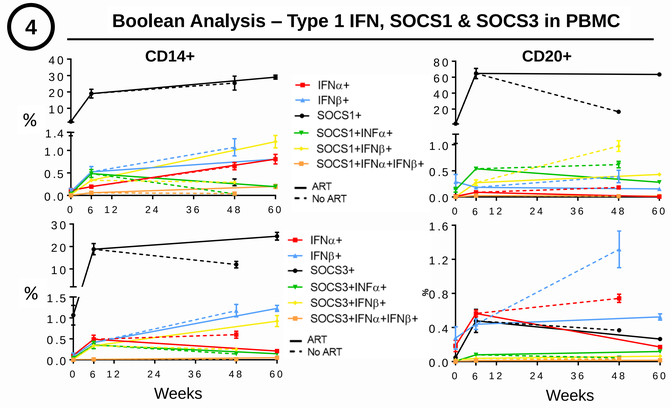
- In untreated animals, SOCS1 and SOCS3 expression significantly declined over time from peak of infection in all populations ($p<0.02$), except for SOCS1 in CD14+ cells, despite persistent and increasing viremia and IFN production, reveling a severe inefficient negative feedback of IFN production.
- In ART-treated animals, SOCS1 and SOCS3 remained stable in all populations, except for SOCS1+/CD8+ and SOCS3+/CD14+ that increased over time ($p<0.002$), despite suppression of viremia, reflecting more closely the IFNβ profile than the IFNα.
- SOCS1 week 60 values in ART+ animals were significantly higher than those at week 48 for untreated animals ($p<0.001$) except for CD14+ where they were comparable.
- SOCS1 and SOCS3 expression percentages were lower in LN than in PBMC in all subpopulations, except for:
  - SOCS1 in CD14+ monocytes (comparable to PBMC),
  - SOCS3 in B cells, where the percentage was higher in LN than in PBMC, and in CD14+ cells where the percentage was comparable in LN and PBMC.

SOCS expression is induced during treated or untreated SIV infection but is insufficient to abrogate IFN expression. This inefficiency is of higher magnitude when the virus burden is higher as it is in the untreated infection. The percentage levels of SOCS1 expression are also lower in LN than in PBMC, possibly reflecting a higher residual SIV expression at this site compared to blood when viremia is suppressed.



- Activation of TLR7 and TLR8, critical to SIV recognition, was not restricted to CD4+ cells that are the major target of SIV infection and is comparable in treated and untreated animals over time. TLR7 expression did not significantly change over time from the levels of peak viremia in treated or untreated animals, indicating that virus replication is not the cause of the observed activation. Surprisingly, it increases significantly in B-cells that are not targets of SIV.
- Lack of change of TLR7 and 8 activation from peak viremia to the later time point in ART-treated animals where viremia is suppressed was unexpected.
- TLR8 expression significantly increased in B cells (CD20) and monocytes (CD14) from baseline while it decreased in T cells, appearing unlinked from viremia.
- Sixty weeks after infection, TLR7 and TLR8 expression in lymph node B cells and CD8+ T cells paralleled what seen in PBMC, while expression of both in CD4 and CD14 was significantly lower than in PBMC. These data also support a stimulation different than SIV in their activation and possibly different than in blood for CD14+ cells.

SIV replication is the initial trigger of TLR7 activation in CD4+ T-cells but its activation cannot be linked exclusively to SIV over the time course of the infection and treatment, particularly in B-cells that are not infectable by SIV and it does not seem affected by ART. TLR8 activation only occurs significantly In CD14+ and CD20 positive cells only. These data support mechanisms different than SIV replication in TLR7 and TLR8 activation.



- In CD14+ monocytes and CD20+ B cells, when SOCS1 or SOCS3 are expressed, most cells do not express Type I IFNs. However, percentages of cells expressing also IFNα or IFNβ, alone or with SOCS1 or SOCS3, are also present, whether the animals were ART-treated or not treated. SOCS3 expression is limited in CD20+ cells.
- While the MFI stayed approximately the same for most of these proteins over time, IFNb increased in % and MFI (not shown).

Although all cells are equally exposed to circulating levels of Type I IFNs, SOCS1 and SOCS3 are expressed in a fraction of the population and this fraction varies in different subpopulations. SOCS1 expression occurs alone in a large proportion of cells, supporting the suppression of Type I IFN production in these e cells. However, in a smaller fraction of cells Type I IFN expression still occurs and SOCS-mediated suppression does not occur. These results support an incomplete negative feedback on Type I IFN production.

CONCLUSIONS

- A dichotomy was observed between expression of IFNα and IFNβ:
  - IFNα seems to mirror viremia in all lymphocytic populations.
  - IFNβ expression occurs predominantly in CD20+ B cells and CD14+ monocytes and increases over time, even when viremia is suppressed, suggesting other sources of stimulation and its independence from SOCS activity.
- Despite all cells being exposed to circulating levels of IFN, only a subset expresses SOCS1 or SOCS3 but not Type I IFNs, suppressing IFN production in those cells. In some cells Type I IFN expression is not suppressed or coexists with SOCS protein expression, indicating a stochastic expression of IFNs and SOCS proteins in the cells.
- Decreased SOCS1 expression over time in most populations of untreated animals, despite high levels of IFN and viremia, reveals a severely inefficient negative feedback. Once induced, SOCS3 expression appears independent of viremia levels and occurs predominantly in CD14+ monocytes.
- Although viremia is reduced and IFNα production decreases accordingly, SOCS1 expression remains stable in ART-treated animals but it is insufficient to reduce levels IFN expression to baseline. The reason is unknown.
- TLR activation remains high, whether the animals were treated with ART or not, pointing at sources outside virus replication for their activation. Microbial translocation is the most likely of them, as it has been shown to persist even with early ART.

ACKNOWLEDGEMENTS

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