HIV inhibits LPS-induced IL-23 and IL-27 production through decreased activation of the p38 and JNK MAPK pathways in human macrophages

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Cytokine dysregulation in HIV infection

Correlated with

- Impaired adaptive immune responses
- Chronic immune activation
  - premature aging/age related diseases
IL-12 family of cytokines

- Link innate and adaptive immune responses

- IL-12 → Th1 responses, HIV-specific CD8 T-cell responses

- IL-12 is decreased in chronic HIV infection
IL-23 and IL-27: Structure and Receptor

Gee et al., 2009. *Inflamm. Allergy Drug Targets*. 8(1):40-52
Objective 1:

Determine whether HIV influences LPS-induced IL-27 or IL-23 production in human MDMs
Does HIV influence LPS-induced IL-27 production in MDMs?

A. HIV inhibits LPS-induced IL-27 EBI3 and p28 mRNA expression, and IL-27 secretion
Does HIV influence LPS-induced IL-23 production in MDMs?

HIV inhibits LPS-induced IL-23 p40 and p19 mRNA expression, and IL-23 p40 and IL-23 secretion.
Objective 2:

Determine the signalling pathways regulating LPS-induced IL-27 production in human MDMs
Does PI3K regulate LPS-induced IL-27?

PI3K positively regulates LPS-induced IL-27 through the p28, and EBI3 mRNA expression
Does p38 MAPK regulate LPS-induced IL-27?

A. EBI3 mRNA fold change

B. p28 mRNA fold change

C. p38 MAPK positively regulates LPS-induced IL-27 through EBI3 mRNA and protein expression
Does JNK MAPK regulate LPS-induced IL-27?

A. EBI3 mRNA fold change

B. p28 mRNA fold change

JNK MAPK positively regulates LPS-induced IL-27 through the p28 mRNA expression and EBI3 protein expression.
Objective 3:

Determine whether HIV inhibits the activation of the signalling pathways regulating LPS-induced IL-27 production
Does HIV influence the activation of the signalling pathways regulating LPS induced IL-27?

A. 

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<th>Mock</th>
<th>HIV</th>
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<tr>
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<td>LPS (mins)</td>
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**p-p38**

**p38**

**Densitometry p38 MAPK normalized to p38 MAPK**

HIV inhibits p38 MAPK and JNK MAPK phosphorylation in LPS-stimulated MDMs

B. 

**HIV inhibits p38 MAPK and JNK MAPK phosphorylation in LPS-stimulated MDMs**

C. 

<table>
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**p-AKT**

**AKT-1**

**Densitometry pAKT normalized to AKT**

HIV inhibits p38 MAPK and JNK MAPK phosphorylation in LPS-stimulated MDMs

NS
Objectives 4:

Determine the signalling pathways regulating LPS-induced IL-23 production in human MDMs
On day 0 post-differentiation, p40 and p19 mRNA expression was induced by LPS however, IL-23 secretion was not consistently observed.

Timeline relative post-differentiation (Days)

-7
0
5
8
15

Production of IL-23 in response to LPS (ie: 100-1000 pg/mL)

Little or no induction of IL-23 in response to LPS

M-SCF differentiation

A. * 

B. *

There is likely a mechanism negatively regulating the secretion of LPS-induced IL-23 in MDMs (ie: translation, secretion mechanism, IL-12 competition for p40)

N=non-responders (N=6)
R=Responders (N=4)
Is LPS-induced IL-23 regulated by the Src pathway

**A.**

LPS-induced IL-23 is negatively regulated by Src pathways in human MDMs

**B.**

**C.**
Conclusion

1. HIV has no effect on basal levels of IL-27 and IL-23 production

2. HIV suppresses LPS-induced IL-27 and IL-23 production in MDMs

3. LPS-induced IL-27 secretion is positively regulated by the PI3K, p38, and JNK pathways in MDMs

4. HIV inhibits p38 and JNK phosphorylation in LPS-stimulated MDMs
   a. Therefore, suppression of p38 and JNK MAPK activation by HIV may be a possible mechanism by which HIV inhibits LPS-induced IL-27 production in MDMs

5. Src negatively regulates LPS-induced IL-23 in MDM
   a. HIV-Nef has been shown to increase Src activation, therefore increased Src activation may be one possible mechanism by which HIV inhibits LPS-induced IL-23 (Trible et al., 2006. J. Biol. Chem. 281:27029-38).
Acknowledgements

Dr. Ashok Kumar and Dr. Jonathan Angel

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University of Ottawa: Admission/Excellence Scholarship
Ontario Graduate Scholarship
NSERC Canadian Graduate Scholarship
OHTN
CIHR

Blood Donors

OGS
HIV-Nef has been shown to activate Src, therefore this may be one mechanism by which HIV inhibits LPS-induced IL-23 in MDMs.
The JNK Are Important for Development and Survival of Macrophages

S. Roy Himes, David P. Sester, Timothy Ravasi, Stephen L. Cronau, Tedjo Sasmono, and David A. Hume

We report in this study that activation of the JNK by the growth factor, CSF-1 is critical for macrophage development, proliferation, and survival. Inhibition of JNK with two distinct classes of inhibitors, the pharmacological agent SP600125, or the peptide D-JNKII resulted in cell cycle inhibition with an arrest at the G2/M transition and subsequent apoptosis. JNK inhibition resulted in decreased expression of CSF-1R (c-fms) and Bcl-xL mRNA in mature macrophages and repressed CSF-1-dependent differentiation of bone marrow cells to macrophages. Macrophage sensitivity to JNK inhibitors may be linked to phosphorylation of the PU.1 transcription factor. Inhibition of JNK disrupted PU.1 binding to an element in the c-fms gene promoter and decreased promoter activity. Promoter activity could be restored by overexpression of PU.1. A comparison of expression profiles of macrophages with 22 other tissue types showed that genes that signal JNK activation downstream of tyrosine kinase receptors, such as focal adhesion kinase, Nck-interacting kinase, and Rac1 and scaffold proteins are highly expressed in macrophages relative to other tissues. This pattern of expression may underlie the novel role of JNK in macrophages. The Journal of Immunology, 2006, 176: 2219–2228.