The Role of LIF Gene in LPS-Induced Pathway

Radwa Elghadafi, Haiam Edabashi, Xiaoren Tang, Yun Ma, and Serge Dibart

Background:

Porphyromonas gingivalis/ lipopolysaccharide (P.g/LPS) induces inflammatory diseases via cytokine production. Our previous data showed that LIF (LPS induced factor) mediates LPS-induced TNF-α production. We found that overexpression of LIF induces Caspase 1/3 in macrophage cells. We hypothesize that LIF may regulate apoptotic proteins/pathways in macrophages.

Aims:

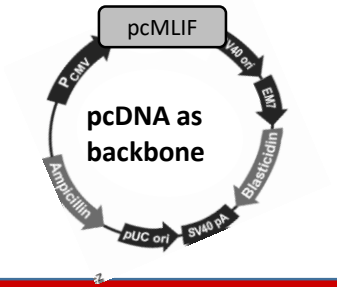
- To determine the effect of LIF overexpression on the production of TNF-α and Caspase1/3
- To develop a nouveau rapid method to analyze the effect of LIF overexpression on known/unknown factors.

Material and Method:

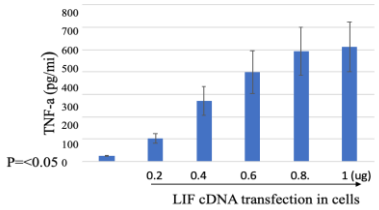
- Mouse macrophage RAW cells
- LIF cDNA cloning and transfection
- ELISA to determine TNF-a, Caspase-1, Caspase-3

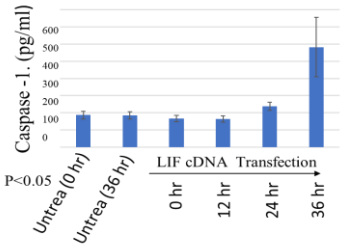
Result:

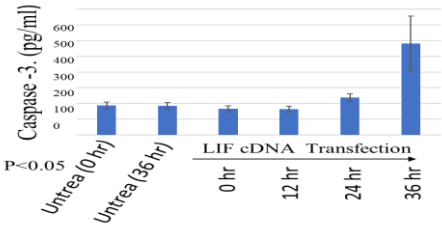
Recombinant LIF DNA



Effect of LIF cDNA overexpression on TNF-a/Caspase 1&3





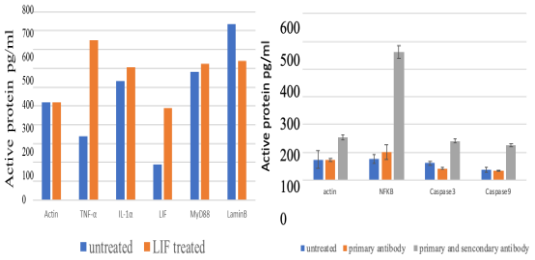


Advantages of our new method (named Websa) compared to western blot

Major processing	Western blot	Our new method	Good point for Our new method
Condition for cell culture	≥80% confluence cells need to be pre-cultured with 2 ml medium in a 6 well plate or 15 ml medium in a 100mm plate for 3-7 days	≥80% confluence cells can be pre-cultured with 0.2-0.5 ml medium in a 96 well plate for 1-2 days	1. Less Cells; 2. Less medium; 3. save time for cell culture; 4. More larger test group/pool available
Cell lysate	Cells should be collected and lysed in an Eppendorf	Cells will be directly lysed in a well of 96 well plate	1. No collection of cells in Eppendorf needed 2. No centrifuge needed for cell collection;
Protein transferred or fixed	Proteins should be run in a SDS-PAGE gel and transferred into filter.	Proteins will be fixed in a well of 96 well plate.	1. Protein tightly fixed on the well of plate 2. No equipment needed to run proteins in SDS-page gel; 3. No equipment needed to transfer protein to filter;
Treatment with 1st Ab and 2nd Ab	Addition of 10 µg Ab / one test / 5ml needed	A minimum amount such as 0.1µg Ab / one test / 0,1ml available	1. Save Ab 2. More larger test group/pool available
Analysis	The signal from filter should be exposed in a film or scanned by image system or developer. Normolization needed for the further analysis.	The signal can be directly and quickly read by reader (Vector 3 or other model reader) with 405/450 absorbance.	1. No image system and developer needed; 2. Data can be easily analyzed by database.

Websa analysis of gene expression

Endogenous **Exogenous**



Conclusion:

1. Overexpression of LIF in RAW cells may induce apoptotic proteins such as Caspase 1/3 and inhibit oral bacteria-induced inflammation.
2. Our Websa saves time and experimental costs. This method can efficiently identify the biological function of multiple genes in one assay.