

# Toll-like Receptor-4 Synthesis is Regulated by JNK Signaling by Three Glucocorticoids Isoforms and by Three Interferons Isoforms, also its Advantages in Activities Depending on the Containment from Arg, Proline, and Hydrophobic Amino Acids in its Compositions

Research Article

Volume 1 Issue 2 - 2021

## Author Details

Ashraf Marzouk El Tantawi\*  
*Biomedical molecular studies, Egypt*

## \*Corresponding author

Ashraf Marzouk El Tantawi, Biomedical molecular studies, Egypt

## Article History

Received: July 13, 2021 Accepted: July 21, 2021 Published: September 24, 2021

## Abstract

Toll-like receptor-4 (TLR4), synthesis is regulated by JNK signaling, by three glucocorticoids isoforms, and by the three interferons isoforms, also depending on availabilities of LPS & on long fatty acids chains with Arg and proline availabilities.

For performing and running the mitochondrial oxidative processes for producing fatty-acyl-CoA-synthetase followed by fatty-acyl-CoA-synthase followed by fatty-acyl-CoA-phospholipase productions for linear TLR4 active beta-subunits which will be transformed into TLR4-alpha upon phospholipase effects, which will follow phosphorylation process (alpha-oxidations) for generate Guanosine triphosphate cyclohydrolase (GTP-Chase) subunits which supposed to contain specific hydrophobic amino acids including Arg, Tyr, leu, proline.... Etc, which is the rate-limiting enzyme for tetrahydrobiopterin (BH4) synthesis, which is essential for inducible iNOS from fatty-acyl-CoA-synthase upon the nitric oxide-synthase (NO-S) regulations effects.

Proline can accelerate anabolic oxidative processes by OPA1 enzymes and provides site-specific flexibility for collagen synthesis in vivo, and also Plays an necessary important roles in TLR4 and TGF-gamma/beta/& alpha synthesis and activities, where the presence of proline in IFN-gamma, in TLR4 genes and in IFN-beta will accelerate oxidative OPA1 anabolic processes and direct the flow of biological processes to proliferations of plasma-membranes, collagen synthesis and blood platelets biosynthesis.

Vitamin E & K-dependent protein C are the key components of anticoagulant serine protease, And therefore vit E and vit k are providing specific advantages to TLR4 synthesis and modulated first in vivo as proper fatty-acyl-CoA-synthetase subunits (gamma-subunits) then modified fatty-acyl-CoA-synthase subunits upon synthase effects on gamma-subunits for producing IL-beta upon which will promote linear TLR4 upon both synthase and phospholipase effects for starting proliferation steps started by catalyzing Arg for producing GTP-Chase and citrulline which the main basis for Erythropoietin productions, for Plasma membrane synthesis... etc.

Both IFN-beta and glucocorticoid-beta are designed anti-inflammatory subunits are depending on each others and on: The activities of OPA1-synthase enzyme for producing the long fatty acyl-CoA-synthase

(Beta-subunit) with specific compositions and sequences from amino acids which can determine their advantages in immunity functions eg their containment of tyr, proline, Arg, gly. etc, where, Both glucocorticoid-beta and IFN-beta are able to recover each others in their different tissues.

**The Purpose of this study:** Understanding the mitochondrial OPA1 inner membrane function in TLR genes synthesis and in the interferons active subunits synthesis and in glucocorticoids isoforms subunits biosynthesis, where both are related to each other and recover each other in vivo.

The presence of proline and Arg amino acids in TLR4 and in IFN-beta will accelerate oxidative OPA1 anabolic processes and will direct the flow of biological processes to proliferations of plasma-membranes, collagen synthesis and blood platelets biosynthesis.

Vitamin E & K-dependent protein C are the key components of anticoagulant serine protease, And therefore vit E and vit k are providing specific advantages to TLR4 synthesis and modulated Anti-coagulations activities and anti-inflammatory functions.

**Materials**

- Interferons (IFN-gamma, IFN-beta, IFN-alpha)
- Glucocorticoid-gamma, glucocorticoid-beta, Glucocorticoid-alpha
- Toll-like receptor (TLR4) genes,
- The three gamma, beta, and alpha-subunits (fatty-acyl-CoA-synthetase, synthase and phospholipase)
- Guanosine triphosphate cyclohydrolase
- Tetrahydrobiopterin (BH4)
- Extracellular linear signal-regulated beta-kinases (ERK1 and ERK2)
- Lipopolysaccharide (LPS), long fatty acids chains
- AMPK
- JNK processes
- FOX genes, and mitochondrial OPA1 inner membrane enzymes
- Glutaric acid to ketoglutarate then to glutamate for proline synthesis (TCA cycle)
- Erythropoietin (EPO)
- Phosphatidylinositol 3-kinase (PI3K)
- S6K1 protein and 23S ribosomal genes

**Introduction**

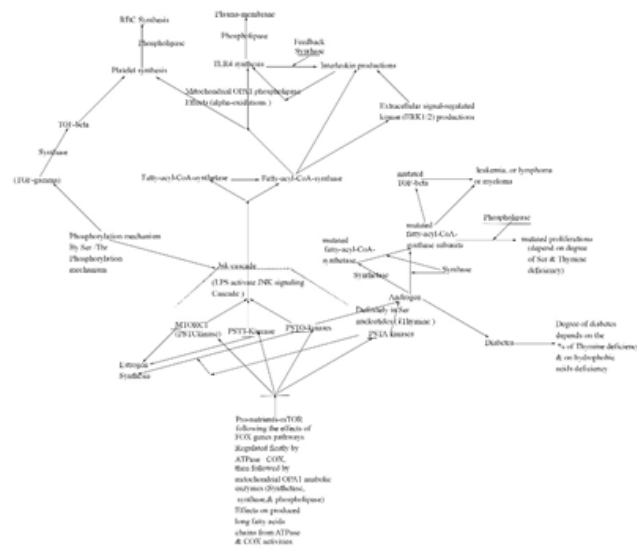
Toll-like receptor-4 (TLR4) biosynthesis is activated by lipopolysaccharide bindings and long fatty acids chains, where LPS is necessary for producing firstly long active fatty-acyl-CoA-synthetase upon the effects of synthetase by activating gamma-oxidations, where, TLR4 biosynthesis can be run through and depending on RORs biosynthesis after FOX genes functions and activities for producing the four kinases molecules forms (where the FOX activities are for producing the four kinds of kinases each carry specific advantages of activities that direct their own functions in biological pathways that later can be identified as mitogens each has its own amino acids in specific qualities and quantities in arranged sequences for advantages of biological processes).

So in Briefly, FOX functions is for preparing and producing specific mitogens mainly in four groups PS/T-kinase, PS/TG-kinase, ps/tt-kinase, & PS/TA -kinase, but second imp steps is functioning some or all the four previous kinases groups for RORs isoforms synthesis which simply are classified into three active long-fatty-acyl-CoA molecules isoforms (Figure 1).

Which formed in so classified arranged three steps each step depending on its previous one for running next one and all steps depending on OPA1 mitochondrial membrane enzymes which presents in specific orders for running their own processes in advantages of highly arranged controlled effects.

Started by gamma-oxidation for producing fatty-acyl-CoA-synthetase (gamma-subunits) upon synthetase effects followed by beta-

oxidations for fatty-acyl-CoA-synthase subunits synthesis which upon phospholipase effects which will promote the TLR4 biosynthesis which also can be stimulated by TCA cycles through firstly stimulating the purines synthesis which will stimulate the synthetase activities for adopting purines productions (by TCA cycles) by pyrimidine synthesis (which run by synthetase for hydrophobic acids production), followed finally by anabolic oxidative processes by OPA1 enzymes for active TLR4 synthesis which through transcriptions will re-activate phospho-lipo-proteins synthesis for phospholipid membrane biosynthesis and cells proliferations.



**Figure 1:** Consider the necessaries of Ser/Thr availabilities in the kinases proteins, and consider the TLR4 synthesis, and TGF-beta synthesis.

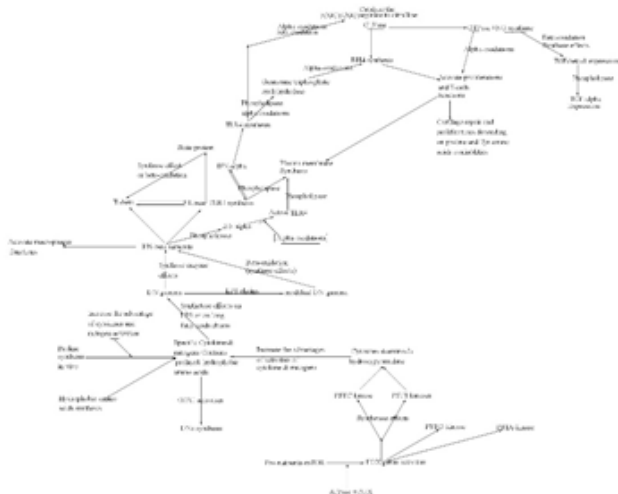
TLR4 functions is the basic tools to Promote the plasma-membrane synthesis and increase its own compositions for signaling receptor activities due to its biosynthesis origin which depend on the four protein kinases compositions and productions started by FOX genes pathways activities (mitogen synthesis) followed by the three anabolic oxidative processes (gamma, beta, alpha-oxidations) by OPA1 enzymes for first producing gamma-subunits then will be oxidized by synthase for producing Beta-subunits which will be oxidized by phospholipase (alpha oxidations) for TLR4 production,

Where, TLR4 is the bases of regulating plasma membrane biosynthesis with specific advantages of compositions, also TLR4 functions is regulating endosome membrane synthesis (which depend on SK6 genes synthesis and on the ribosomes composition) upon the effects of phospholipase on beta-subunits by running for producing active fatty-acyl-CoA-phospholipase subunits for phospho-lipo-protein synthesis and for cells proliferation pathways. TLR4 regulate the MHC class II biosynthetic process, and through its feedback that reactivate and adopt the positive regulation of JNK cascade, where JNK signaling processes are the main basis for TLR4 biosynthesis too and for proliferation pathways (Figure 2).

TLR4 also regulate and adopt the interleukins productions, and Promote the regulation of extracellular linear signal-regulated beta-



kinases (ERK1 and ERK2), For re-activate T-helper 1 cells type immune response, also promote Platelet biosynthesis pathways through interferon beta and then alpha productions (where Interferons considered as cytokines that through transcriptions will innate immune



**Figure 2:** IFN-gamma promote IFN-beta production which is the main regulator for the linear TLR4 biosynthesis which depend on phospholipase for phospholipid and plasma-membranes synthesis.

Response against viral infection and then for types of advantages of cells proliferations), also TLR4

Regulate the NLRP3 inflammasome.

**Methods and Results**

Toll-like receptor-4 (TLR4) biosynthesis started by the Stimulations of mitogen synthesis through FOX functions processes then followed by the effects of the mitochondria OPA1 enzymes on availability of long fatty acids chains or on lipopolysaccharide bindings for producing the three types of active subunits isoforms started by gamma subunits upon the effect of synthetase for producing acyl-CoA-synthetase then followed by effect of synthase on gamma-subunits for producing beta-subunits (fatty-acyl-CoA-synthase subunits) then ended by productions of fatty-acyl-CoA-phospholipase (alpha subunits) upon the effects of phospholipase on Beta-subunits, where long fatty acids chains and LPS production *in vivo* is first upon the effects of ATPase and COX enzymes on lipid, and on lipoproteins which are necessary for activating the productions of firstly the long active fatty-acyl-CoA-synthetase followed by producing the fatty-acyl-CoA-synthase then followed by fatty-acyl-CoA-phospholipase (alpha-subunits) productions upon mitochondrial OPA1 enzymes effect, where the alpha-subunits productions is the necessary step for TLR4 synthesis including specific containment from specific amino acids which imp for increasing anti-inflammation, for phospholipid synthesis (Figure 3).

TLR4 can promise the regulations of linear extracellular signal beta-kinases (ERK1/2) that through feedback can re-activate gamma-kinases gamma-subunits by gamma-oxidation and further cellular dependent activities which participates in the regulations of many cellular activities including adopting purines results from TCA cycles, and adopting pyrimidines synthesis *in vivo* by synthetase for hydrophobic acids synthesis, Where both TCA cycles and synthetase activities are cooperating well together for providing the proper purines and pyrimidines for building the proper gamma-subunits (long fatty-acyl-CoA-synthetase) during gamma-oxidation by synthetase effects, and those two cycles are related to each other and considered necessary for adopting purines and pyrimidines synthesis *in vivo*.



**Figure 3:** Both Glucocorticoids isoforms subunits & IFNs (IFN-gamma, IFN-beta & IFN-alpha) are having the responsibility of recovering each other's & GTPase productions for BH4 synthesis during cellular cycles *in vivo*.

**JNK signaling cascade regulate TLR4 synthesis depending on phospholipase effects**

c-Jun N-terminal kinases (JNKs), identified as subunits proteins kinases where produced firstly by FOX genes activities pathways (where FOX functions are specific for mitogen proteins kinases family synthesis). JNK processes depends on FOX genes functions followed by OPA1 enzymes Effects on the produced mitogen from FOX genes activities.

The JNK activation occurs through a dual phosphorylations mechanism of threonine (Thr) and Ser /Tyrosine (Ser/Tyr) residues within Protein-S/T-Thymine-kinase (Thymine kinases) and on mTORC1 (Protein-S/T-Cytosine-kinases which considered P13k Akt) subdomain.

The JNK module considered as the basis for adopt, promote, and running proliferations through the mitochondrial OPA1 anabolic oxidative processes which run in highly arranged specific regulations by its enzymes for producing the three types of fatty-acyl-CoA gamma, beta, and alpha active subunits for running anti-inflammatory pathways, for proliferations processes started by TLR4 synthesis, for re-activating T-cells, and for introducing proper anti-inflammatory processes through modifying and increasing specified cytokine productions, that JNK module steps are basically depending basically on the FOX gene for producing the four kind of protein kinases that depend firstly on the phosphorylation of Ser /Thr mechanism for stimulating and running the necessary mitochondrial anabolic oxidative processes started by gamma-oxidation, followed by beta-oxidation and ended by alpha-oxidations where the last is specified for proliferations started by TLR4 synthesis which depend on phospholipase effects on linear beta-kinases (beta-subunits) which through feedback and transcription will reactivate linear extracellular regulated kinases ERK1/2.

The JNK is necessary in the regulation of platelet biosynthesis, interleukin-1 productions, interleukin-12 productions, interferon-gamma, beta, and specifically interferon-alpha (IFN-alpha) productions.

The functions and activities of FOX genes are basically specify for producing the four kind mitogens of kinases that those mitogen kinases will follow the JNK function passway under the effects of anabolic mitochondrial OPA1 enzymes regulations with the availabilities of LPS or long fatty acids molecules chains for producing TLR4 genes, cytokines, for IFNs back productions and for cells proliferation steps.

JNK-interacting protein 3 associates with Toll-like receptor 4 and is involved in LPS-mediated JNK activation [1].

Where, the protein kinases that produced from FOX activities pathways will follow the effects of mitochondrial anabolic enzymes



through JNK with LPS availabilities for TLR4 synthesis upon first running gamma-oxidation by synthetase effects on LPS, then followed by beta-oxidation on gamma-subunits through the effects of synthase for producing linear extracellular signal-regulated beta-kinases (ERK1 and ERK2) which upon phospholipase will re-activating the TLR4 synthesis and activities, T-helper 1 type immune response, and Platelet biosynthesis pathways through interferon alpha productions.

Phorbol ester is long fatty-acyl-CoA-synthetase active subunits are produced due to effects of synthetase enzyme on long fatty acids chains, which will follow the effect of synthase for producing the long-fatty-acyl-CoA-synthase active subunits which considered as the basis of extracellular signals- regulated beta-subunits which necessary for activating the TLR4 synthesis, and for increasing of interferon-beta (Interferon-1) productions [2].

Mitogen-activated protein kinases (MAPK) are basically produced by ATPase and COX followed by FOX functions:

MAPK stored or located on human chromosomes but originally formed through FOX genes activities regulated by ATPase and OPA1 activities where, MAPK encodes kinases proteins of amino acids regulated by mitochondrial OPA1 enzymes and by ATPase activities.

MAPK is proteins kinases that characterized by the presence of specified amino acids (in specific qualities and quantities in specific arrangements) In addition to the importance of presence of the serine / threonine is for running specific phosphorylations mechanism on those two amino acids for Brocken and separating their triplets nucleotides {{ TCT – Ser

TCC – Ser

TCA – Ser

TCG – Ser

ACC – Thr

ACA – Thr

ACG – Thr}} for producing the four types of basic kinases : 1st, protein-Ser /Thr -Thymine kinases (PSTT-K)

2, protein-Ser /Thr -guanine kinases (PSTG-k)

3, protein-Ser /Thr -Cytosine kinases (PSTC-k or mTORC1)

4, protein-Ser /Thr -adenosine kinases (PSTA-k)

Notice the main necessary nucleotide in Ser is the Thymine nucleotides where deficiency of Thymine will lead deficiency in PSTT-K and PSTC-k productions and will reflect decreasing in estrogen synthesis with increasing in androgen (where the main nucleotide in androgen is adenosine with almost absence of thymine nucleotides), that will reflect diabetic health problems.

### MAPK are mainly and formed by FOX activities in main four kinds of kinases

Produced during the activities and functions of FOX genes that will follow the mitochondrial anabolic enzymes effects (synthetase, synthase, and phospholipase) for modifying the produced four kind of protein kinases PSTGk, PSTCk, PSTTK, and PSTA Kinase, where their functions and their advantages of specificities depends on their amino acids composition and their arrangement in specific sequences, which will appear *in vivo* as specific mitogen that necessary for reactivating RORs isoforms synthesis and for producing the three Fatty Acyl-Coenzyme-A isoforms (gamma, beta, and alpha) for running specified cycles for anti-inflammation processes and for cellular proliferation processes.

The MAK productions by FOX genes activities can follow the JNK signaling cascade upon the effects of mitochondrial anabolic enzymes for activating mitochondrial anabolic oxidative processes for the TLR4 biosynthesis in the availabilities of Lipopolysaccharide (LPS) and long fatty acids chains (LFC),for TLR4 synthesis for S6K protein synthesis, and for NF-κB production which depending on the three necessary active-lipo-proteins subunits (isoforms) productions upon OPA1 activities :

- a. Fatty-acyl-CoA-synthetase (gamma-subunits)
- b. Fatty-acyl-CoA-synthase (beta-subunits)
- c. Fatty-acyl-CoA-phospholipase (alpha-subunits).

The advantage of origin, quality, quantity, of each amino acids are characterizing the mitogen kinases subunits functions and activities, and hormones composition activities, Where, the availabilities of proline (C<sub>5</sub>H<sub>9</sub>NO) which can be produced from TCA cycles *in vivo* “and from the conversion of glutaric acid to ketoglutarat then to glutamate” can activate Hippo pathways and TLR4 synthesis by promoting the OPA1 anabolic oxidative processes (gamma-oxidation and beta-oxidations upon synthetase then synthase respectively) which needed for TLR4 productions, for the transforming growth factor-beta (TGF-β) productions, and for interleukin-beta production, where TGF-β1 productions started firstly by TGF-gamma productions upon first the effects of synthetase enzyme on long fatty acids chains, on LPS, and on fatty phospho-lipo-proteins (which produced from ATPase and COX oxidative activities for producing prostaglandins), then will be following the effect of synthase enzyme on fatty acyl-CoA-synthetase for producing proper fatty-acyl-CoA-synthase, which considered as beta-subunits (with specific composition of amino acids) that produced by beta-oxidation (synthase effects) that necessary for Interleukin-beta production and for TGF-β productions for adopting cellular and extracellular-signals regulated kinase (ERK1/2)activities, for adopting anti-inflammatory processes and then for running the cells proliferations upon the phospholipase effects on IL-beta and on Beta-subunits.

Proline, Arg, and hydrophobic amino acids eg Tyr, and Leu are so imp to be involved in IL-beta and in TLR4 composition:

It's true that Proline does not contain the amino group -NH<sub>2</sub>, but is containing active -ve oxygen linkages that can stimulate and promote ATPase and stimulate gamma-oxidation easily followed by beta-oxidations, then alpha-oxidation, and at the main time able to reactivate the idle inactive +ve linkages (sub-fragments) in kinases proteins molecules for specific modified mitogen protein kinases productions through FOX genes functions, then followed by OPA1 activities, where the effects of OPA1-synthetase enzyme is carry the function of hydrophobic acids synthesis by the effects of synthetase enzyme through running Gamma-oxidations for creating specific active gamma-subunits, followed by the effects of synthase for producing active beta-subunits through running beta-oxidations for producing Beta-subunit for the further synthesis of alpha-subunits through alpha-oxidations effects by phospholipase effects on Beta-subunits where alpha oxidations are so necessary for cells proliferation, for phospholipid bio-synthesis, for promoting T-cells synthesis, and for cells proliferations processes.

The synthesis of proline *in vivo* is so imp for adopting purines synthesized from TCA cycles, where proline is related to and depending on the synthetase activities for pyrimidine synthesis, and in the main time stimulate synthetase activities and Thymine synthesis is for adopting the production of purines (from TCA cycles) upon the synthetase effects on long fatty acid chains, and also proline biosynthesis *in vivo* is so necessary for reactivating macrophages and



T-cells where due to the conversion of glutamate to ketoglutarate then to glutamate results of the consuming NH for creating and producing NO-S upon synthase effects (beta-oxidation) which is imp for reactivating macrophages, and also necessary for hydroxy-proline productions.

The hydroxyproline is an accurate index of collagen content and production as well as normal lung tissue [3]. Also, Proline provides *in vivo* site-specific flexibility for collagen [4].

Proline plays an necessary important roles in TLR4 and TGF-beta/alpha synthesis and activities, that the presence of proline in TLR4 and in TGF-beta will possess specific advantages in cellular processes and will direct the flow of biological reactions to collagen and blood platelets synthesis, where proline plays imp functions in plant growth and in collagen flexibility, that availabilities of proline in TLR4 genes and in TGF-beta will accelerate gamma-oxidation, then beta, then alpha-oxidations for alpha-subunits production that will give flexibility to tissues, blood platelet, and collagen synthesis.

The Proline biosynthesis *in vivo* is a determinant of imp keys of many cells membranes and proteins activities, where also proline plays important roles in increasing signals activities, in increasing anabolic oxidative processes by OPA1 enzymes, and in increasing plants developments through their cells walls developments.

The proline biosynthesis *in vivo* is depending on TCA cycles functions and on the availabilities of glutatarate and succinyl-CoA, where, the role of the production of hydroxyproline- and the proline-rich proteins are important for the characterizing cell-membranes proteins structures and proline play pivotal roles in cell signal transduction activities throughout characterizing the ERK1/2 activities and TLR4 biosynthesis .

Decreasing in Ser amino acids and deficiency in proline synthesis *in vivo* lead to decreasing in pyrimidine synthesis, that will not adopt the activities of TCA cycles which produce purines, and in many cases can lead to decreasing in vit D that will reflect less absorption in intestine. Proline imp for producing soluble Cytosine nucleotides Linked actively to each of other for cytokine production (CCC, CCG, CCA. CCT) which have mainly advantages to migrations characteristics and increase cytokine activities. Availability of two Cytosines in cytokines are so imp for the migrations characters of molecules and for cytokine synthesis result of the formation of cytosine- 4-amino-2-hydroxypyrimidine, Cytokines including the chemokines, interferons (INFs), interleukins, lymphokines, and tumor necrosis factors (TNF), are Basically, contains hydrophobic amino acids chains for contains the kinases produced by FOX genes pathways activities where some of those kinases produced from FOX actually can be linked together for producing specific kind of cytokine contain advantages for proper cellular activities, which will follow specific pathways for feeding cells and immune through the Mitochondrial OPA1 gene proper activities, where those kinases or mitogen can produced back by a range of cells activities including immune cells like macrophages, B lymphocytes, T lymphocytes and mast cells, as well as endothelial cells, fibroblasts, and various stromal cells.

The advantages of the presence proline in Cytokines molecules are promoting the maturation, cells growth, and responsiveness of particular cell populations but through mitochondrial regulations and modification for producing gamma-subunits then beta-subunits upon effects of synthetase and synthase respectively.

I consider that the tissues that produce cytokines are the tissues of the FOX genes activities for producing the four kinases proteins, where cytosine, guanine, and thymine proteins kinases are the basis of imp necessary active protein kinases for cytokines biosynthesis, for INFs biosynthesis, for antigen synthesis and for IL-beta productions.

The deficiency in proline biosynthesis and in pro-nutrients-mTOR will reflect decreasing in cytokine proper activities, (where proline can promote the formation of cytosine- 4-amino-2-hydroxypyrimidine which can increases the molecules migration and their flexibilities and their proper activities).

Lead to decreasing in the anabolic oxidations and increase the pathogenic mutations in IFNs molecules, lead to the Revival the viral emissions *in vivo*.

Cytokines can be modified "improved" in the Golgi (but not synthesis in Golgi) that as I mentioned before that cytokines are basically depending on FOX functions followed by OPA1 enzymes activities, and their advantages depending on the quality and quantity of specific amino acids in their active subunits, where cytokines can traffic through the endoplasmic reticulum as soluble mediators (specifically if contain proline), and they remain to modulate membranes binding and membrane functions, and they can be produced into cytosolic active forms that can be migrated for further modification by mitochondrial OPA1 activities, that can be returning to nucleus that can act as transcriptional regulators for promoting mitochondrial oxidative processes: firstly Gamma-oxidations then by beta-oxidation respectively by OPA1 enzymes functions.

Where, Cytokines bind to reciprocal receptors which prepared originally by OPA1 enzymes for producing the fatty-acyl-CoA-subunits (isoforms) receptors that reside either on the membrane of a target cell or in the soluble phase Membrane receptors, and can drive novel gene expression to promote effector functions plus can increase host defenses by up-regulating and improvement the antigen presentation, but through either the protein amino acids subunits or through the structure of fatty-acyl-CoA gamma or beta then alpha-active linear subunits.

The cytosine- 4-amino-2-hydroxypyrimidine (C4a2hP) is basically its synthesis depend on two kinases protein which are cytosine-Ser /Thr kinases (Protein-S/T-Cytosine-kinase) and protein-Ser/Thr-Thymine-kinase (PSTT-Kinase), where the (C4a2hP) considered as the main basics of IFNs compositions and main for protein C components, where deficiency in (C4a2hP) will lead to deficiency in IFNs activities and in protein C biosynthesis lead to decreasing or deficiency in the alveolar epithelium as a dynamic barrier in pathogenesis.

IFN-gamma promote IFN-beta production upon synthase effects which is the main regulator for linear TLR4 biosynthesis which depend on phospholipase for phospholipid membranes synthesis.

The biosynthesis of IFNs started by IFN-gamma which dépend on synthetase effects followed by IFN-beta (which dépend on synthase) then IFN-alpha (which depend on phospholipase) respectively, where last beta and alpha-oxidations are the mirror of re-activating TLRs genes synthesis, where IFN-alpha enhanced TLR responsiveness in macrophages by up-regulating the expression of TLR3, TLR4, and TLR7 [5].

Interferon-alpha (IFN alpha) and interferon-gamma (IFN gamma) each induced in susceptible target cells a state of resistance to viral replication and can reduced cellular proliferation, presumably through different mechanisms [6].

Interferons are basically cytokines depending on FOX genes and followed by effect of mitochondrial OPA1 enzymes functions, where after cytokine production upon FOX activities will be modified by the effects of mitochondrial enzymes to produce specific modified IFN-gamma with specific amino acids hydrophobic acids in specific sequences upon synthetase enzymes, then will be followed the synthase effects on IFN-gamma for producing modified IFN-beta (upon the effects of synthase) which will confer its resistance in functions to viral infections and considered to be imp for linear TLR4



synthesis, then will follow alpha oxidations for producing IFN-alpha (upon phospholipase effects) which has the responsibility for TLR4 biosynthesis, where each of IFN structure has own steps in functions and activities, and all IFN subunits isoforms structures are depending on each others for strengthen and running immune activities for anti-inflammation activities and for potentiate macrophages and T-cells activities. where, Interferons are related cytokines identified by their ability to confer resistance to viral infections [7].

The Defective IFN- $\beta$  signaling (may due to decreasing in proline, in Arg or in necessary hydrophobic amino acids) are having the responsibility for Lower Ability of BALB/c Macrophages to Produce NO in Response to LPS [8].

The availabilities of proline and other necessary active hydrophobic amino acids in IFN-beta subunits molecules are so imp and responsible for determining the advantages of IFN- $\beta$  activities and TLR4 biosynthesis too (which produced upon phospholipase effects).

But at the main time the values of amino acids in quality and in quantity containment in each IFN isoform subunits can determine its own advantages in activities of its own subunits functions depending on its composition from proline, Arg, and from necessary hydrophobic amino acids, that each IFN subunit can re-start its own basic activity for reproducing its own active subunits chain, then will start to activate the other two IFNs subunits again, eg: in IFNs biosynthesis when IFN- $\beta$  increases will increase its own beta-oxidations that will increase the Antiviral effect then will potentiate the macrophages activities (but in availability of proline, Arg and necessary hydrophobic acids), then will activate and potentiate the TLR4 synthesis and IFN-alpha activities in orders, while the re\_increasing in IFN-gamma will increase its own gamma-oxidation for its own modified gamma-subunits reproduction that can cause a temporary suppression to other two IFNs producers and to TLR4 synthesis and will reactivate macrophages activities [9], due to the IFN-gamma activities will increase its own gamma-oxidation for analyzing membranes and inflammations for reactivating firstly pyrimidine synthesis for hydrophobic acids synthesis for reproducing its own new modified IFN-gamma subunits (in availability of LPS) and for adopting purines and pyrimidines *in vivo*, that upon synthase enzyme (beta-oxidation) will re-activate IFN-beta subunits productions which can potentiate macrophage activity and anti-inflammation processes, then upon phospholipase effects on IFN-beta will activate alpha oxidations for IFN-alpha productions which will reactivate TLR4 synthesis (also depend on phospholipase effects), which has the anti-inflammatory function too and can reproducing IFN-beta again throughout the feedback upon OPA1-synthase enzyme effects (through feedback of beta-oxidation).

The protein C system provides important functions and control of blood coagulation where proteins C basically are the necessary regulator for fatty-acyl-CoA-synthetase synthesis and for IFN-gamma-subunits productions upon synthetase effects. That both Vitamin E & K-dependent protein C are the key components of anticoagulant serine protease. And therefore vit E and k are modulated first *in vivo* their own necessary proper gamma-subunits synthesis (fatty-acyl-CoA-synthetase subunits) which is the basics for producing IL-beta upon the synthase effects which will regulate TLR4 synthesis upon the phospholipase effects then will produce BH4 upon beta and alpha oxidations for reactivating phospholipid membranes synthesis and at the same time act for anticoagulation [10].

The presence of vitamin K in the IFNs production for TLR4 gives its advantages for anti- coagulation characters and possess its own cells the ability (which contain vitamin K and vit E in their plasma membrane and contains proline and Arg amino acids in their composition) to resist and prevent blood clotting and dissolve it *in vivo*.

Protein C (APC) has a protective effect on mechanical tension and barrier integrity in human alveolar epithelial cells (A549) exposed to thrombin [11].

The respiratory epithelium, in particular, is a primary target of an inflammatory/infectious condition at the epithelial-blood interface and is itself capable of amplifying an inflammatory signal for stimulating IFN-gamma and IFN-beta productions, where the IFN-beta will firstly potentiate macrophages activities and anti-inflammatory processes the reactivate IFN-alpha production which is the first necessary for cells proliferations.

That inflammatory signals can stimulate IFN-gamma activities then OPA1 functions for restart the gamma, beta, then alpha-oxidative processes upon effects of synthetase, then synthase then phospholipase respectively for re-producing IFN-gamma, IFN-beta and for IFN-alpha subunits productions respectively, then for TLR4 synthesis respectively and the b for BH4 synthesis for recover broken cells membranes and for proliferation.

As I mentioned before that IFN-gamma alone can suppress both macrophages and TLR4 synthesis and activities even can suppress their production from interleukin (IL-beta), that as IFN-gamma. Started to be activated as IFN-gamma oxidations will start for analyzing inflammations and available content of molecules for creating their own modified gamma-subunits which contains advantages of specific amino acids in arrangements for recreating step of beta-oxidation for producing the (Beta-subunit) IFN-beta subunits upon the synthase effect which will be responsible for re-creating the linear TLR4 which will be responsible for BH4 synthesis and then for Plasma membrane synthesis upon phospholipase effects.

Where, In sharp contrast, IFN- $\gamma$  was shown to synergize with TLR agonists for induction of macrophage tumoricidal activity and production of both of both NO and pro-inflammatory cytokines (TNF- $\alpha$ , IL-...), furthermore, IFN- $\gamma$  was shown to suppress macrophage IL-10 secretion induced by TLR agonists [12].

Phospholipase is the basic regulated tool for TLR4 synthesis which act on IFN-beta subunits, for producing TLR4, where both are sharing together the responsibility for producing growth factor BH4 for proliferations.

As soon as TLR4 synthesized and the effect of phospholipase continued as the alpha-oxidations process will begin and continued for the Releasing of Guanosine triphosphate Cyclohydrolase (GCHase) molecules which supposed to contain specific hydrophobic amino acids including proline, where Guanosine triphosphate. Cyclohydrolase considered as the rate-limiting enzyme for tetrahydrobiopterin (BH4) synthesis and reactivities, which considered as the growth factor tools for cells proliferation and for T-cells activations, That, The metabolite BH4 controls T cell proliferation in autoimmunity [13].

In other way and other clear meaning that the synthesis of TLR4 in proper active structures with availability of proper phospholipase are the basic tools for growth factor BH4 productivity, where its synthesis is depending on to beta-oxidation followed by alpha-oxidations phosphorylation processes on Arg amino acids for taking guanine nucleotides for releasing Guanosine triphosphate Cyclohydrolase (GTP-Chase) and release citrulline where the GTP-Chase is necessary for BH4 modulations.

At deficiency in GTP-Chase synthesis will lead to dysfunction in BH4 production, where Patients with recessive GTP-CH deficiency demonstrate much more neurologic dysfunction than the dominant form, with early-onset developmental delay, seizures, tremors, truncal hypotonia with appendicular hypertonia, and autonomic dysfunction [14].



I would like to add that deficiency in amino acids which contains Guanine nucleotides in their triplets as Arg, cys, leu, Ala, Val.. will lead to deficiency in the GTP-Chase productions and may can strongly be related to deficiency in TCA cycles which necessary for purines production.

Both IFN-beta and glucocorticoid-beta are designed anti-inflammatory subunits are depending on the activities of synthase enzyme within OPA1 mitochondrial membrane for producing the long fatty acyl-CoA-synthase (Beta-subunit) with specific compositions and sequences from amino acids that can determine their advantages in immunity functions. Both glucocorticoid-beta and IFN-beta are able to recover each other's in their different tissues, that Glucocorticoids Conditioning of Myeloid Progenitors can Enhance TLR4 Signaling [15].

### Stimulated Glucocorticoid-beta

Subunits in a proper biological environment *in vivo* can stimulate and recover TLR4 synthesis and then the BH4 proliferations factor in the availabilities of proper active sequences of OPA1 synthase and phospholipase, where the both pervious enzymes can reactivate the beta-oxidations and alpha-oxidations necessary for producing GTP-Chase and for reactivating P13K which is necessary for BH4 synthesis, where beta and alpha-oxidations can catalyze Arg (and other amino acids which contain guanine) for using their guanine nucleotides for producing GTP-Chase enzymes (and citrulline) for enhancement the TLR4 productions and then for BH4 productions for proliferation processes.

Also, promoter of the human glucocorticoid receptor (hGR) gene contains an interferon (IFN) regulatory factor element (IRF-E) [16], that at recovery of IFN, the RF-IFN will be activated first by transcriptions then through beta-oxidations for reproducing IFN-beta which under effect of alpha-oxidations will recover the TLR4 synthesis for necessary proliferations, for plasma membrane synthesis and for macrophages and T-cells reactivation.

Netrin-1 is a combination of glucocorticoids (gamma and beta) with IFN-beta and IFN-alpha active subunits with TLR4 where, YAP signaling pathway is depending on firstly TAZ pathways through the production of the gamma subunits which will be the basis of the beta-subunits production upon the effects of synthase enzyme, glucocorticoids gamma and beta subunits are so necessary for netrin-1 synthesis for neutrophile repairs and for brain functions where netrin-1 are carry imp rules in anti-inflammatory effective processes due to their origin basically are glucocorticoids-gamma and then glucocorticoid-beta.

The YAP (fatty acyl-CoA-synthase activities) Pathway is reflecting a critical role of the effect of Glial cell line-derived neurotrophic factor (GDNF) against A $\beta$ -induced inflammatory response in microglia [17].

Glucocorticoids subunits (glucocorticoid-gamma & beta) are acting as activating factor for the GDNF and for the membrane receptors DCC activations, where both glucocorticoids subunits (gamma and beta) are considered as the basic receptors for re-activating IFN gamma, for IFN-beta, and then for netrin-1 synthesis which considered is having so imp roles for neuronal signals migration basically for neuron and brain functions.

Netrin-1 can follows the re-effect of synthase enzyme for Dynamic trafficking of a-amino-3-hydroxy-5-methyl4-isoxazolepropionic acid glutamate which is the basis for proline synthesis, and for GTP-Chase synthesis which is the basis for BH4 synthesis necessary for cells proliferation including brain functions. proline and Arg are so imp for GTP-Chase and then for BH4 synthesis which dependent basically on P13k which produced from FOX functions & from OPA1 anabolic enzymes.

Erythropoietin is glycoprotein synthesized in the liver, but it looks like its production switches almost exclusively to the kidney, but facts ensue that Erythropoietin is filtered and re-extracted through renal tubules, where red blood cells carry wast toxic molecules including positive molecules to the kidney, where Erythropoietin need to be re-extracted by renal tubules in kidney due to its importance and values in its compositions to cells proliferations.

As Erythropoietin decreased as BH4 biosynthesis in liver will be increased for recover the decreased Erythropoietin, that will reflect increasing in TLR4 re-activities and production and increasing in GTP-Chase productions which result from catalyzing the Arg (CGG, CGA) amino acids for releasing GTP-Chase enzymes & the citrulline (that I consider citrulline is originally cytosine nucleotides which basically bonded to guanine in Arg triplets) which will be used for reactivating the Akt (p13k) for reproducing Erythropoietin again.

Where, re-activation of phosphatidylinositol 3-kinase (PI3K) is important for erythropoietin-induced erythropoiesis from CD34(+) hematopoietic progenitor cells [18]. Now, one of the important role for PI3K (which has been modified by synthase and by phospholipase through beta-oxidations and alpha-oxidations respectively) is the reactivating and accelerating TLR4 for re-producing BH4 which is necessary for EPO-induced survival, proliferation, and maturation of early erythroid progenitors.

Also, the site of 23S rRNA ribozyme, 0, was both necessary and sufficient to trigger TLR13-dependent interleukin-1 $\beta$  production [19]. That P13K are basics of S6K and 23S ribosomal genes productions and repairs which are carrying necessary roles in TLR synthesis and 8n BH4 productions with advantages composition from necessary active amino acids.

### Conclusion

Toll-like receptor-4 (TLR4), synthesis is regulated by JNK signaling, by three glucocorticoids isoforms, and by the three interferons isoforms, also depending on availabilities of LPS & on long fatty acids chains with Arg and proline availabilities.

Proline can accelerate anabolic oxidative processes by OPA1 enzymes and provides site-specific flexibility for collagen synthesis *in vivo*, and also plays an necessary important roles in TLR4 and TGF-gamma/beta/& alpha synthesis and activities.

Phosphatidylinositol 3-kinase (PI3K) is important for erythropoietin-induced erythropoiesis reactivations, and we have consider that P13K are basics of S6K and 23S ribosomal genes productions and repairs which are carrying necessary roles for TLR synthesis and 8n BH4 productions with advantages of containment of necessary active amino acids eg: proline, Arg, leu, tyr...etc.

Both IFN-beta and glucocorticoid-beta are designed anti-inflammatory subunits are depending on each others and on the activities of OPA1-synthase enzyme for producing the long fatty acyl-CoA-synthase

(Beta-subunit) with specific compositions and sequences from amino acids which can determine their advantages in immunity functions eg their containment of tyr, proline, Arg, gly.. etc, where, Both glucocorticoid-beta and IFN-beta are able to recover each others in their different tissues, and in almost general fact the IFNs are related to glucocorticoids subunits and are covering each other *in vivo*.

The containment of Arg, and prolines amino acids in genes and in active fatty-acyl-CoA-subunit isoforms including glucocorticoids and interferons three subunits can accelerate anabolic OPA1 oxidative processes and can save living cells and their tissue from hypoxia.

### Conflict of Interest

The Author declare that the research work has been conducted in the



absence of any commercial or financial Relationships, that could be construed as a potential conflict of interest.

## References

1. Tetsuya Matsuguchi, Akio Masuda, Kenji Sugimoto, Yoshiyuki Nagai, Yasunobu Yoshikai (2003) JNK-interacting protein 3 associates with Toll-like receptor 4 and is involved in LPS-mediated JNK activation. *MBO J* 22(17): 4455–4464.
2. I Momose, M Terashima, Y Nakashima, M Sakamoto, H Ishino, et al. (2000) Phorbol ester synergistically increases interferon regulatory factor-1 and inducible nitric oxide synthase induction in interferon-gamma-treated RAW 264.7 cells. *Comparative Study* 1498(1): 19-31.
3. Kehrer JP, Lee YC, Solem SM (1986) Comparison of in vitro and in vivo rates of collagen synthesis in normal and damaged lung tissue. *Exp Lung Res* 10(2):187-201.
4. Chow WY, Forman CJ, Bihan D, Anna MP, Rakesh R, et al. (2018) Proline provides site-specific flexibility for in vivo collagen. *Sci Rep* 13809(2018).
5. Sirén J, Pirhonen J, Julkunen I, Matikainen S (2005) IFN- $\alpha$  regulates TLR-dependent gene expression of IFN- $\alpha$ , IFN- $\beta$ , IL-28, and IL-29. *J Immunol* 174(4): 1932-1937.
6. Levy DE, Lew DJ, Decker T, Kessler DS, Darnell JE (1990) Interaction between interferon- $\alpha$  and interferon- $\gamma$  through induced synthesis of one subunit of the transcription factor ISGF3. *EMBO J* 9(4): 1105-1111.
7. Panel JJ Graber, S Dhib-Jalbut (2014) Reference Module in Neuroscience and Biobehavioral Psychology. *Encyclopedia of the Neurological Sciences (Second Edition)*. 2014: 718-723.
8. Oliveira LS, de Queiroz NMGP, Veloso LVS, Moreira TG, Oliveira FS, et al. (2014) A Defective TLR4 Signaling for IFN- $\beta$  Expression Is Responsible for the Innately Lower Ability of BALB/c Macrophages to Produce NO in Response to LPS as Compared to C57BL/6. *PLoS ONE* 9(6): e98913.
9. Kang K, Bachu M, Park S, Kang K, Bae S, et al. (2019) IFN- $\gamma$  selectively suppresses a subset of TLR4-activated genes and enhancers to potentiate macrophage activation. *Nat Commun* 3320(2019).
10. Booth SL, Golly I, Sackeck JM, Roubenoff R, Dallal GE, et al. (2004) Effect of vitamin E supplementation on vitamin K status in adults with normal coagulation status. *Am J Clin Nutr* 80(1): 143-148.
11. Puig F, Fuster G, Adda M, Blanch L, Farre R, et al. (2013) Barrier-Protective Effects of Activated Protein C in Human Alveolar Epithelial Cells. *PLOS ONE* 8(2): e56965.
12. Elisabeth Müller, Panagiotis F Christopoulos, Alexandre Corthay (2017) Toll-Like Receptor Ligands and Interferon- $\gamma$  Synergize for Induction of Antitumor M1 Macrophages. *Front Immunol*.
13. Cronin SJF, Seehus C, Weidinger A, Talbot S, Reissig S, et al. (2018) The metabolite BH4 controls T cell proliferation in autoimmunity and cancer. *Nature* 563(7732): 564-568.
14. Daniel M. Raben (2013) Phosphofructokinase-2/Fructose Bisphosphatase-2. *Encyclopedia of Biological Chemistry*. pp: 456-458.
15. Tian Y. Zhang and Raymond A. Daynes (2007) Glucocorticoid Conditioning of Myeloid Progenitors Enhances TLR4 Signaling via Negative Regulation of the Phosphatidylinositol 3-Kinase-Akt Pathway. *J Immunol* 178(4): 2517-2526.
16. Nunez BS, Geng CD, Pedersen KB, Millro-Macklin CD, Vedeckis WV (2005) Interaction between the interferon signaling pathway and the human glucocorticoid receptor gene 1A promoter. *Endocrinology* 146(3): 1449-1457.
17. Jie Qing, Xiaoheng Liu, Quan Wu, Mengjie Zhou, Yuwei Zhang, et al. (Hippo/YAP Pathway Plays a Critical Role in Effect of Glial cell-derived neurotrophic factor (GDNF) Against A $\beta$ -Induced Inflammation in Microglial Cells. *DNA and Cell Biology* 39(6): 1064-1071.
18. Myklebust JH, Blomhoff HK, Rusten LS, Stokke T, Smeland EB (2002) Activation of phosphatidylinositol 3-kinase is important for erythropoietin-induced erythropoiesis from CD34(+) hematopoietic progenitor cells. *Exp Hematol* 30(9): 990-1000.
19. Xiao-Dong Li, Zhijian J Chen (2012) Cell biology, Immunology, and Inflammation, Sequence specific detection of bacterial 23S ribosomal RNA by TLR13. *Cell Biology Immunology and Inflammation* 1: e00102.

