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Regulation of indoleamine dioxygenase by polyunsaturated fatty acids-interfering with cancer mediated immunosuppression

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Introduction

- Evasion from the normal immune response is essential for any cancer cell to persist
- One mechanism by which cancer cells suppress the immune response is through modulating the level of Tryptophan

Tryptophan (Trp)

- Tryptophan is the least abundant of all of essential amino acids
- Trp is used in protein synthesis and is a precursor of key biological molecules including melatonin and serotonin
- Cells are acutely sensitive to changes in its local concentration

Metabolism of Tryptophan

- Trp can be degraded by two enzymes
- Hepatic Tryptophan 2,3-dioxygenase (IDO) & extra-hepatic, Indoleamine 2,3-dioxygenase (IDO)
- IDO controls the level of Trp during inflammation
- Metabolism of Trp leads to the production of Kynurenine (Kyn) metabolites which have immunotoxic and immunosuppressive activity

Indoleamine 2,3-dioxygenase (IDO)

- IDO is an important immune control enzyme
- Cells that express IDO
 1. Are capable of suppressing local T cell responses
 2. Promote immune tolerance under important physical and pathological conditions such as foetal rejection, infectious diseases, organ transplantation, inflammatory disorders and **cancer**

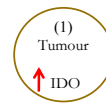
Distribution of IDO

- IDO is widely distributed in most cells
- Highest expression/activity is in Antigen presenting cells which regulate the immune response

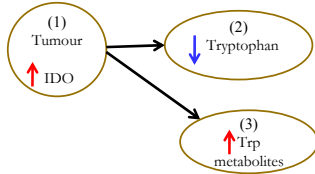
IDO expression in cancerous cells

- Nearly all primary tumour-cells express elevated level of IDO while normal cells of the same stroma were negative for IDO expression
- Tumours that express high levels of IDO are not rejected by a pre-immunized host
- Inhibition of IDO by the competitive inhibitor, 1-methyl-tryptophan leads to tumour rejection
- Patients with tumours that express high levels of IDO have a low survival rate (numerous studies)

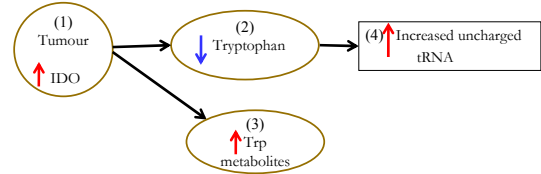
IDO mediated tumour-induced tolerance



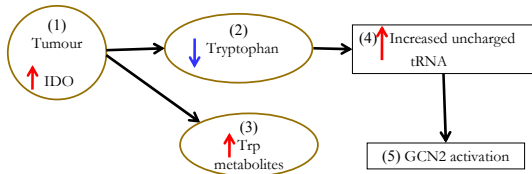
IDO mediated tumour-induced tolerance



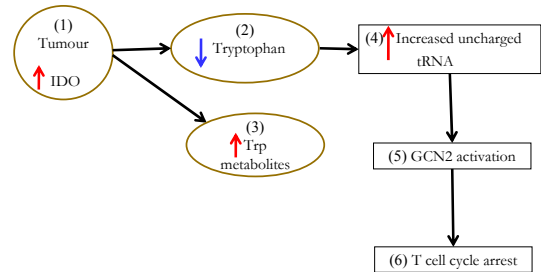
IDO mediated tumour-induced tolerance



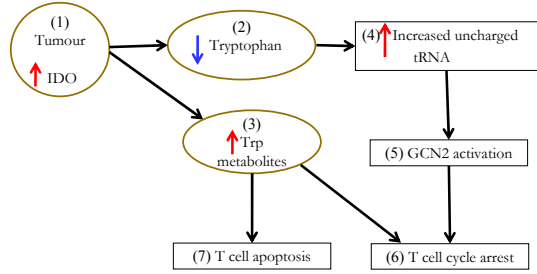
IDO mediated tumour-induced tolerance



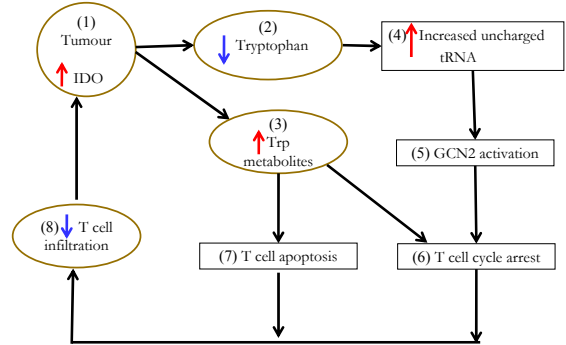
IDO mediated tumour-induced tolerance



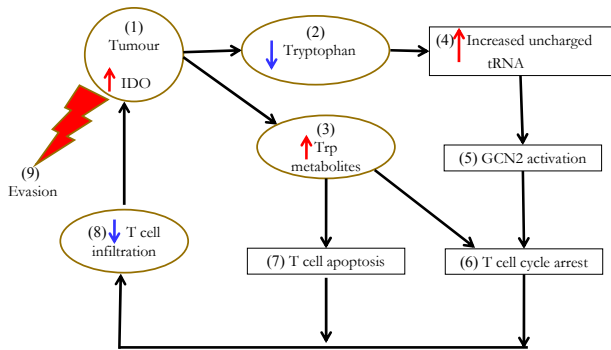
IDO mediated tumour-induced tolerance



IDO mediated tumour-induced tolerance

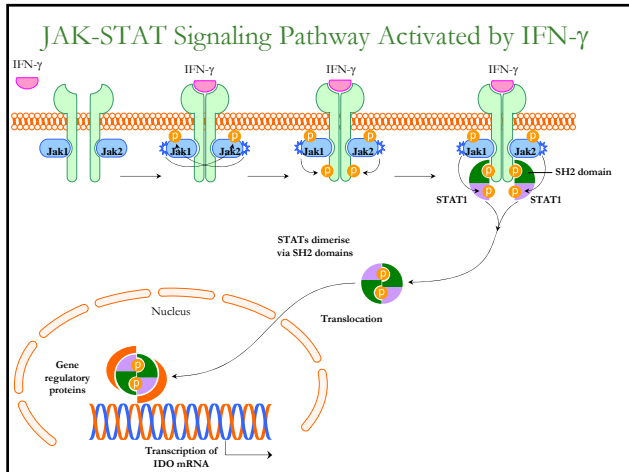


IDO mediated tumour-induced tolerance



Induction of IDO

- The main physiological inducer of IDO is IFN- γ which acts via JAK1/2-STAT1 signalling pathway.



Induction of IDO

- Another inducer of IDO is PGE₂
- PGE₂ is derived from the metabolism of the Polyunsaturated fatty acids; Arachidonic acid (AA) via the cyclooxygenase pathway
- AA, has been shown to modulate the immune response
- However the role of AA on IDO activity has not been investigated

Arachidonic acid (AA; 20:4 n -6)

- AA is present in membrane phospholipids
- Upon its release, AA can be metabolised into a number of biologically active eicosanoids
- The biological activities of fatty acids are affected by chain length, number of double bonds and series (ω -3 vs. ω -6)

Metabolism of AA

- AA $\xrightarrow[\text{COX1 \& COX2}]{\text{Cyclo-oxygenase (COX)}}$ Thromboxanes (e.g. TX₂) + Prostaglandins (e.g. PGE₂)
- AA $\xrightarrow[\text{(LOX)}]{\text{Lipoxygenase}}$ Leukotrienes (e.g. LTB₄)
- AA $\xrightarrow{\text{Cytochrome P450}}$ Epoxy acids.

Relationship between AA and IDO

- AA $\xrightarrow{\text{COX}}$ PGE₂ (pro-inflammatory)
- PGE₂ + TNF \longrightarrow \uparrow IDO (in maturation of DCs)
- Inhibition of COX pathway \longrightarrow \downarrow IDO

Summary

- \uparrow IDO in cancer cells \longrightarrow progression
- \downarrow IDO in cancer cells \longrightarrow rejection
- AA $\xrightarrow{\text{COX}}$ PGE₂
- PGE₂ (Pro-inflammatory) \longrightarrow \uparrow IDO
- Inhibition of COX \longrightarrow \downarrow IDO

Hypothesis

- AA is likely to increase IDO production/activity in cancer cells through a PGE₂ dependent mechanism

Aims

- Determine the effect of AA on IFN- γ induced IDO activity in THP-1 cells
- Determine the mechanism by which AA modulates IDO activity

Methods

- IDO activity was estimated indirectly by measuring Kyn
- Kyn was measured using two methods:
 1. Colorimetric assay
 2. HPLC

Colorimetric assay

- Is an absorbance based method
- Aromatic amino group of Kyn reacts with Ehrlich's reagent
- Ehrlich's reagent forms a Schiff base reaction
- Has the advantage of being high throughput, rapid, sensitive and inexpensive

HPLC

- Commonly used method for detecting Kyn and Trp
- It is very specific, but slower and more expensive than the colorimetric assay
- We used HPLC to:
 1. Compare the sensitivity and the specificity of the two methods.
 2. Confirm the results obtained by the colorimetric assay

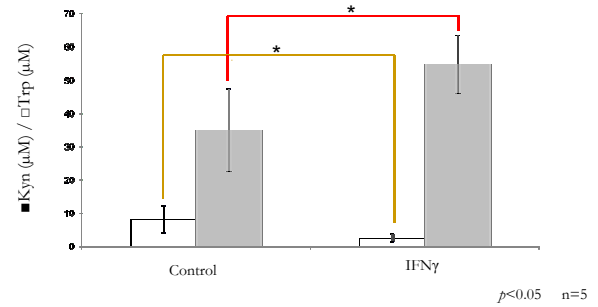
Results

Comparison of colorimetric and HPLC methods

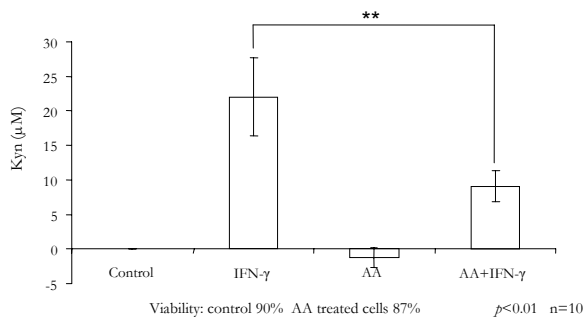
Kynurenine (μM)	Colorimetric Mean \pm SEM	HPLC Mean \pm SEM
10	11.5 \pm 2.4	9.9 \pm 0.8
20	21.5 \pm 2.1	24.7 \pm 4.0
40	33.8 \pm 2	32.2 \pm 1.3
80	78.9 \pm 4.2	76 \pm 3.9

$p > 0.05$, n=10

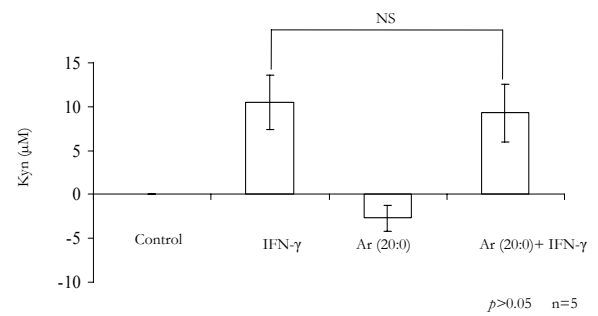
Changes in Try and Kyn levels following treatment with IFN- γ (1000IU/ml)



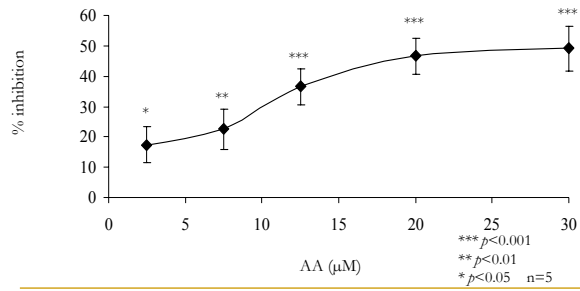
Effect of AA (20 μM) on IFN- γ induced IDO activity



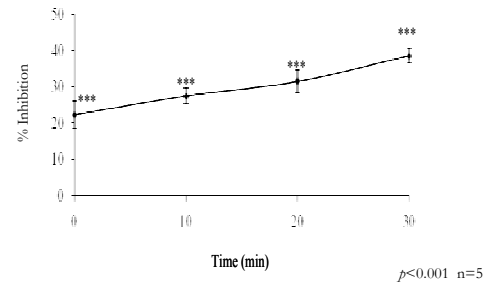
Importance of Unsaturation: effect of Arachidic acid (20:0) on IFN- γ induced IDO activity



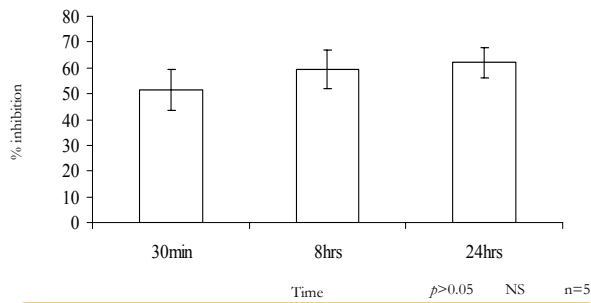
Effect of AA concentration on IFN- γ induced IDO activity



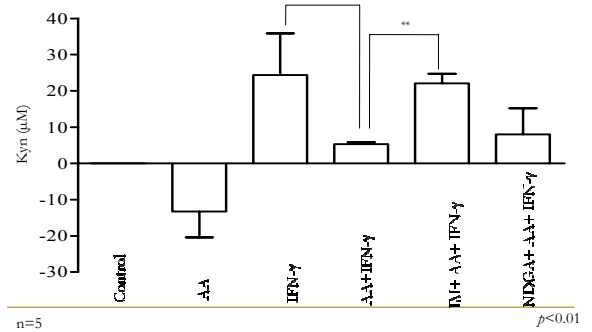
Inhibition of IDO activity is mediated rapidly



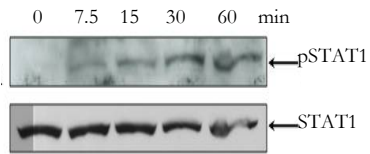
Incorporation of AA is not important for activity



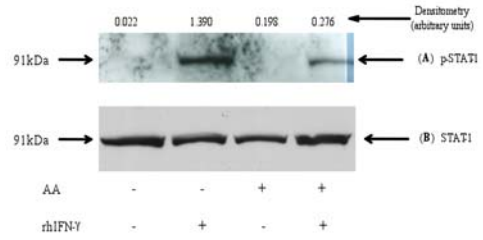
Blockage of the COX but not LOX pathway prevents the activity of AA



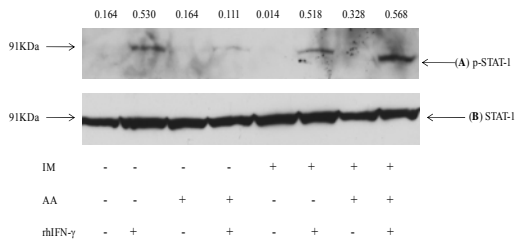
Kinetics of STAT-1 phosphorylation by IFN- γ



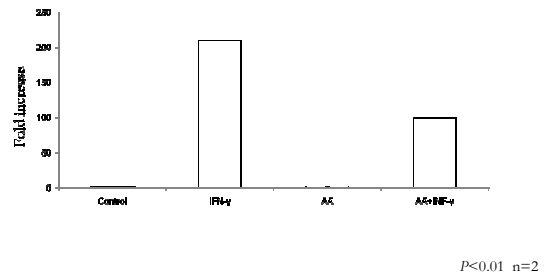
AA inhibits IFN- γ mediated STAT1-P

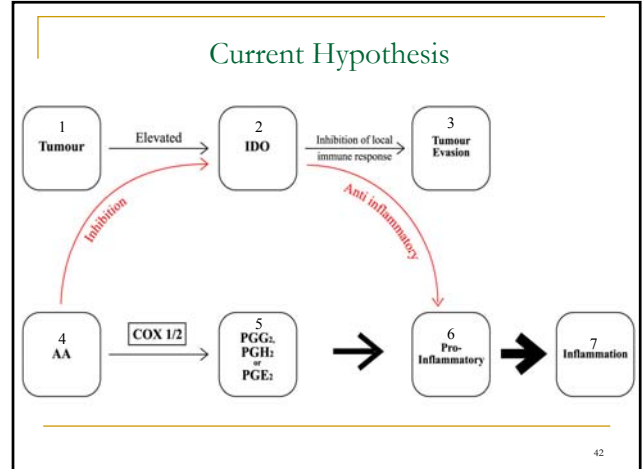
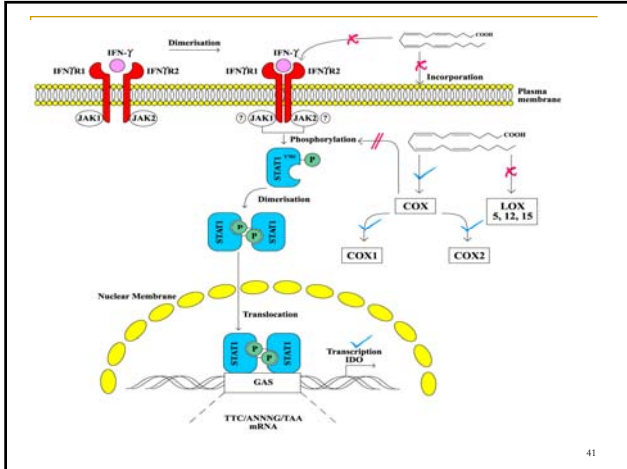


A COX metabolite of AA inhibits STAT-1 phosphorylation



AA inhibits IDO mRNA production





Future studies

- Identify the bioactive metabolite(s)
- Determine the effect of the active metabolite(s) on
 - Cell surface receptors
 - JAK1/2 phosphorylation
 - STAT-1 phosphorylation
 - IDO mRNA expression and protein expression
 - IDO enzyme activity

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