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Ozone versus human health - a review

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Abstract : Ozone is a triatomic form of Oxygen. Its molecular formula is O_3 and is found in stratosphere. It extends from about 6 Km at poles, 17 Km at equator & 50 Km above the earth's surface. In normal condition, it is present in the atmosphere at about 0.5 ppm at sea level, though variations from an average of 0.02 ppm in winter to about 0.07 ppm in summer have been noted.

When ultra violet light strikes an oxygen molecule, the photon splits the oxygen molecule into highly reactive oxygen atoms, these readily combine with an oxygen molecule & forms Ozone.

$$O2 \xrightarrow{\text{UV light}} 20^{-} + 2H$$

$$O^{-} + O2 \longrightarrow O_3$$

Ozone on the one hand protects the lives on earth surface from the lethal and harmful effects of UV-β radiations emerging from Sun's rays by providing a giant umbrella which filters out radiations below 3000 A^0 and controls the thermal budget yet acts as an ubiquitous urban pollutant causing damage to alveolar epithelium. Inhalation of ozone to a tune of 0.1 to 0.2 ppm is sufficient to cause toxicity leading to edema, hyperresponsiveness, impaired host defensive mechanism, psoriasis, skin cancers, increase in the level of malondialdehyde (MDA) in the skin etc. Exposure to ozone results into the release of archidonic acid eicosanoids (Prostaglandins and their related compounds prostacyclin PGI, thermoboxanes TXA, leukotriens LT & lipoxins are collectively called eicosanoids.) and platelet activating factors from a variety of pulmonary cell types reports Samet *et al* (1992) and Mekinnon *et al* (1995).

Acute inhalation of Ozone is associated with an inflammatory response showing accumulation of macrophages at the injury site. The macrophages and alveolar epithelial cells get activated & release cytotoxic and pro-inflammatory mediators.

In this review the author has compiled informations from the works of Laskin *et al* (1998) and Cross *et al* (1998) and few other workers and explained how pro-inflammatory cytokines like TNF-α (tumor necrosis factor), interleukin (IL-Iβ) and bacterially derived LPS (Lipopolysaccharide) singly or jointly modulate the cellular function by shifting the oxidation reduction equilibrium or modulate the regulatory protein which initiate gene transcription. The author has also dealt with how NF-β transcription factor binding to: NOS gene is the root cause of $\dot{N}O$ production responsible for pulmonary toxicity leading to inflammation (via JAk-STAT pathway).

Keywords: Ozone; Damage; Alveolar cells; Skin.

INTRODUCTION

Ozone is a triatomic form of oxygen when UV light strikes an Oxygen molecule, the photon splits the oxygen molecule into two highly reactive oxygen atoms. These readily combine with an oxygen molecule & form ozone. In the absence of any such reaction O_3 settles into a dynamic steady state in which the rate of its formation is equal to the rate of its destruction. It is destroyed when it reacts with hydroxyl radical ($\dot{OH}$) & perohydroxyl species (HO_2) which plays a role in photochemical smog formation.

In concentration less than 2ppm it has pleasant smell but irritating when higher. Ozone layer at a height of

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12-56 km above the earth's surface in *stratosphere*, makes a giant shield and protects plant and animal world from the lethal effects of UV/β radiations and controls the thermal budget yet acts as an ubiquitous urban air pollutant causing various types of damages: Such as:

1. damage to alveolar epithelium.
2. Inhalation of O_3 to a tune of 0.1 to 0.2 ppm causes toxicity leading to edema, airways hyper-responsiveness, impaired host's defensive mechanism (i.e., immune system) Lippman, 1989.
3. Psoriasis and skin cancer.
4. Triggers hypersensitivity response analogous to NADPH– oxidase system of animal phagocytes called oxidative burst Dwyer *et al* (1996)[1].
5. cataract, malignant melanoma.
6. It has been reported by Samet *et al* (1992)[2] and Mckinnon *et al* (1993)[3] that variety of pulmonary cell types release *arachidonic acid*, *eicosanoids** and platelet activating factors on exposure to ozone.

Lavnicova *et al* (1998)[8] and Pendino (1993[5]) too also reported that ozone induces inflammatory reaction marked by rapid accumulation of neutrophils and macrophages and type II epithelial cells showed enhanced cellular activity and released $\dot{NO}$ and reactive oxygen intermediates. Macrophages also produced pro-inflammatory cytokines interleukin (1L)1β and tumour necrosis factor alpha (TNF–$\propto$) – Pendino *et al* (1995)[9]

Marletta (1993)[10] opines that $\dot{NO}$ is produced from the terminal guanidine nitrogen of L-arginine with the help of NO synthase. **(Fig.1)** $\dot{NO}$ on one hand plays an important role in haemodynamic changes in septic and hemorrhagic shocks and plays an important role in biological systems (Palmer *et al* 1987[11], Ignarrow *et al*, 1987[12]) viz, vasodilation (Furchgott *et al,* 1980[13]) Platelet functions (Rodomski *et al,* 1987[14]), neurotransmission (Snyder, 1992[15], Bredt *et al,* 1989[16]); metalloproteins in certain enzymes interact with and modulate their activity, combines with iron heme of soluble guanylate cyclase leading to the formation of cyclic guanosine monophosphate (cGMP) which in turn increase cellular action of (like relaxation of smooth muscle fibers, aggregation and adherence of platelets & haemotaxis in neutrophils yet higher concentration of is toxic and aggravate cell injury or arthritis (Evans *et al,* 1996[17]), Lung diseases, Laskin *et al,* 1994[18]): *atherosclerosis* Mathys *et al,* 1997[19]).

High concentration of $\dot{NO}$ interferes with DNA leading to its fragmentation, during inflammation it reacts with superoxide anion radical () and yields peroxynitrite ($OONO^-$) a toxic oxidant (Beckmann *et at*, 1993[20]) which decays in acidic condition and produce hydroxyl like free radical (Beckmann *et al*, 1990[21]). ($OONO^-$) is a long lived oxidant (Beckmann *et al,* 1993[20]) which initates lipid peroxidation and which in the presence of other lung irritants, injures lung tissue and brings alveolar epithelial damage & reacts with sulhydral group of cell membrane (Radi *et al*, 1991[22]) and modifies structural protein through nitration of tyrosine residue (Hadded *et al*, 1994[22]) though ($OONO^-$) shows cardioprotective and cytoprotective effects after it combines with S–nitrosylate glutathion or other thiol containing substances and forms S-nitrosylate (Lefer *et al*, 1997[23])

Acute inhalation of ozone cause accumulation of *neutrophils* and *macrophages* at the site of alveolar cell injury called inflammatory response due to which oxygen intermediates and $\dot{NO}$ are formed Pendino *et al* (1993[5]) and Punjabi *et al,* (1994[6]) leading to lung injury (Fig.2).

*Eicosanoids – *Prostaglandin* and their related compounds – *Prostacyclins* (PGl), *thromboxanes* (TXA), *leukotrienes* (LT) and *lipoxins* are collectively called *eicosanoids*.

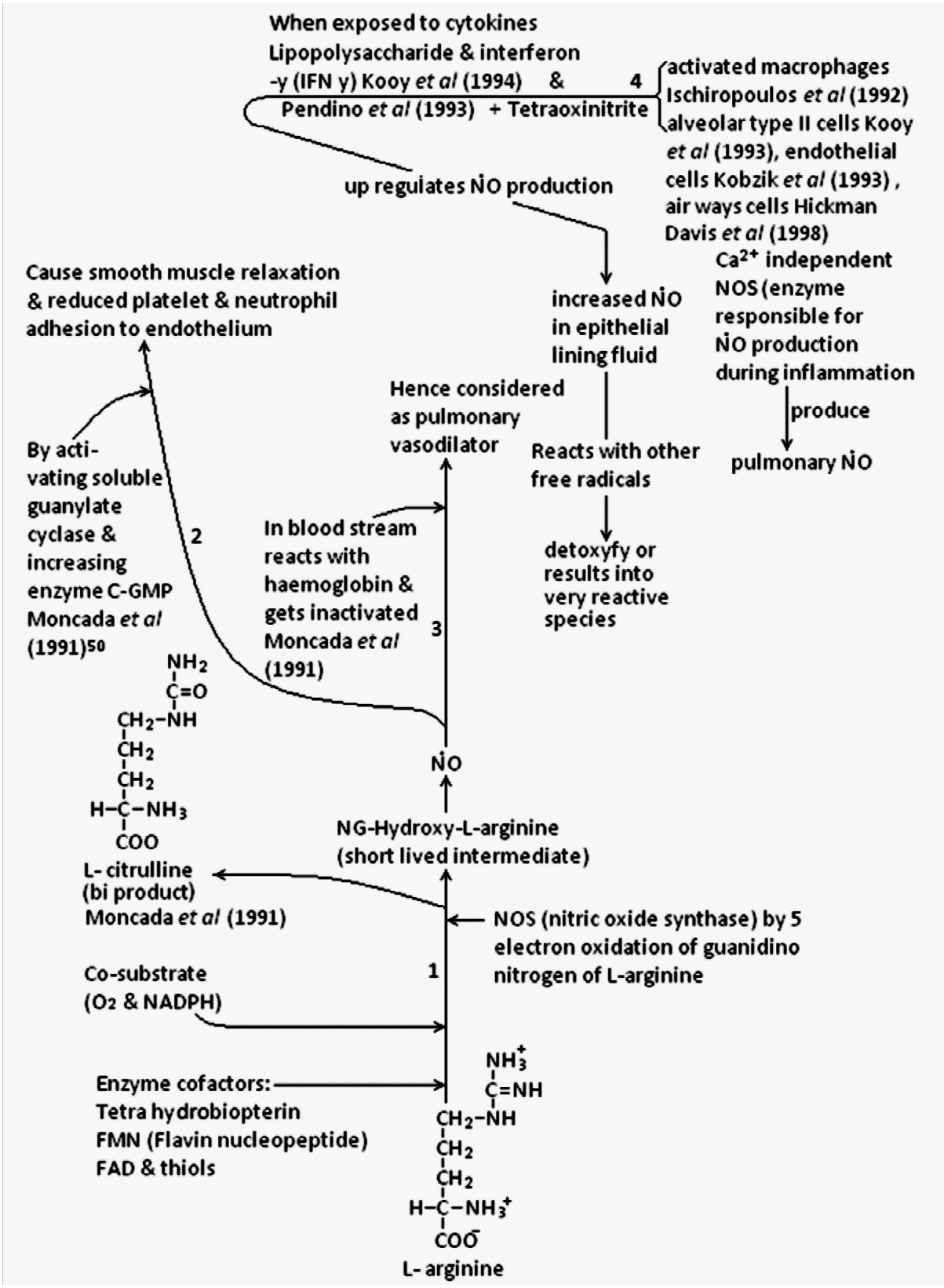

Fig.1. ṄO from L-arginine & what it causes

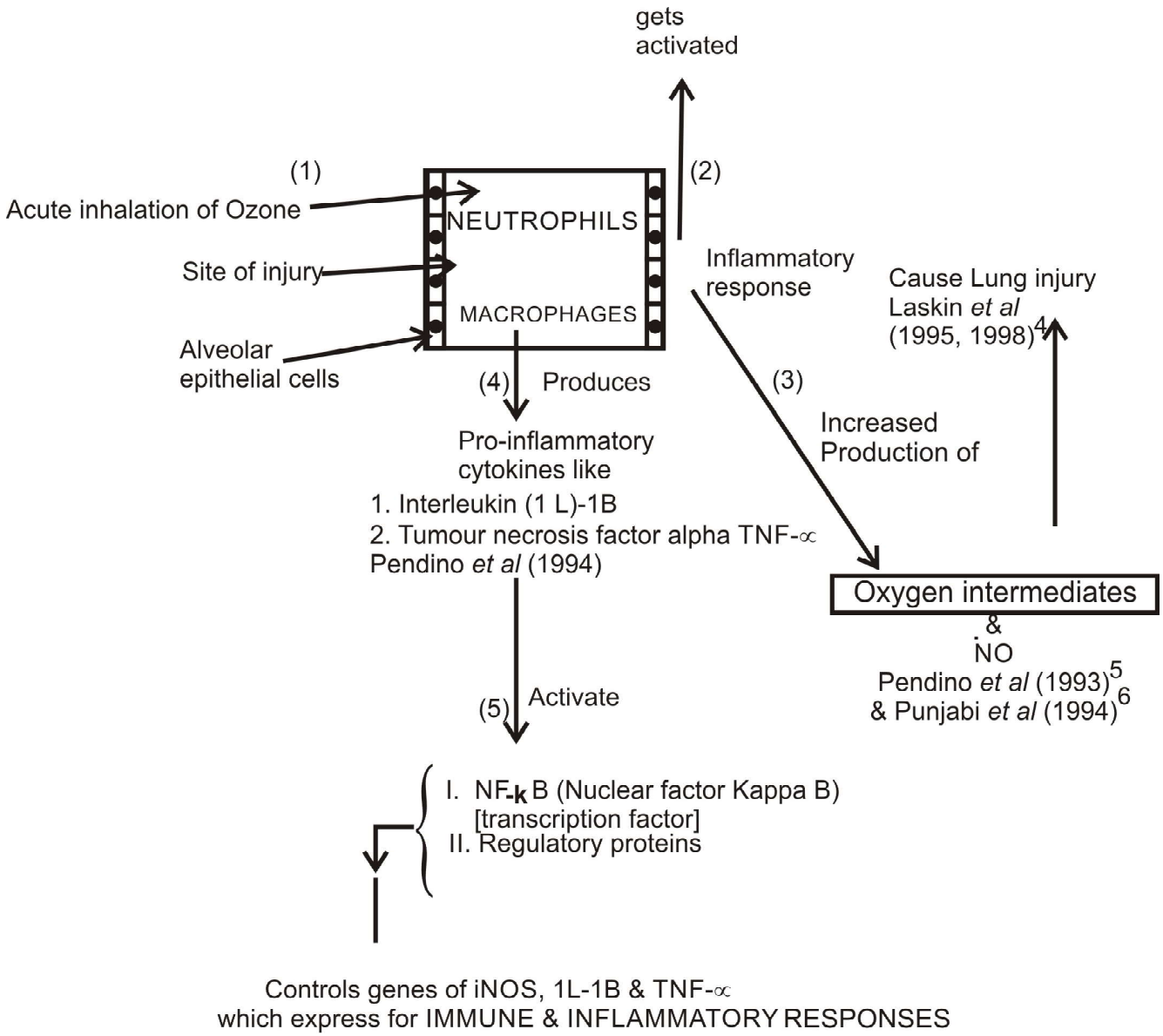

Fig.2. Consequences of acute inhalation of ozone

The macrophages in particular produce Pro-inflammatory cytokines like interleukin (1L) – 1β and tumour necrosis factor alpha (TNF∝) - Pendino *et al*, (1994, 1995)[7,9]

These Pro-inflummatory cytokines in turn activate nuclear factor Kappa B ($NF-_kB$) – a transcription factor and regulatory proteins which control the genes of (iNOS, 1L 1β TNF) & subsequently express for immune and inflammatory responses.

Laskin *et al,* (1998)[4b] exposed pathogen free sprague Dawley female rats kept in air tight plexiglass chamber in which ozone concentration was maintained by adjusting the UV light and flow rate of ozone (amount of ozone being 2ppm for 3 hours) via ozone monitor. They isolated cells from the lung after 3 to 48 hours using suitable methods as under (**fig.-3 and table-1**). Cells were kept in culture media & assessed by suitable methods for $\dot{NO}$ production, iNOS protein expression in cell lysate, visualisation of proteins & nuclear proteins (**table II**).

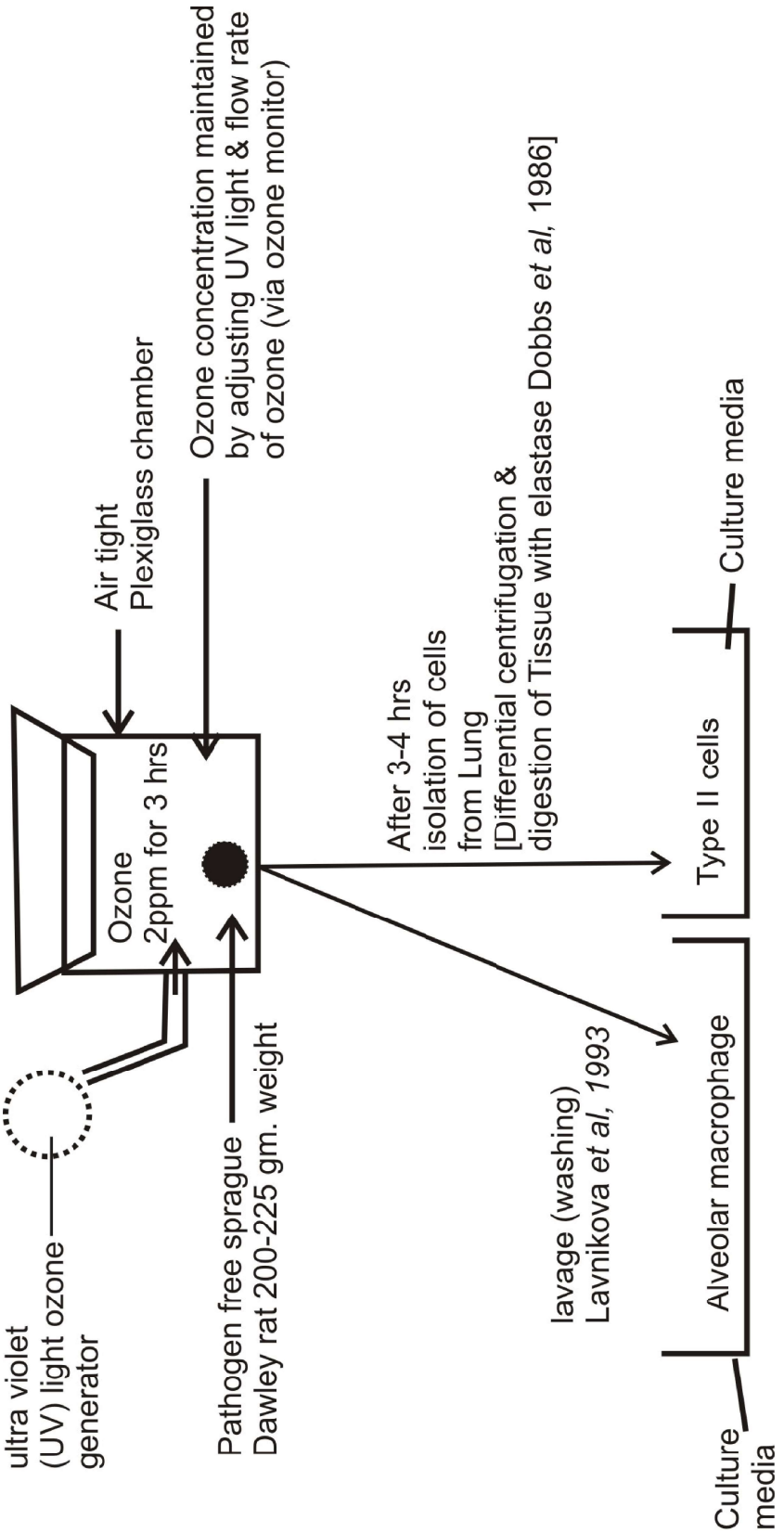

Fig.3. Isolation of cells from Lung after Ozone inhalation.

The methods employed by Laskin *et al* (1998)[4b] to assess the presence of $\dot{NO}$, iNOS protein expression and presence of nuclear protein as in Tab. I & Tab II

Table – I

Type of cells	Method employed
Alveolar macrophages	by washing or Lavage as suggested by Lavnikova *et al* (1993[8]).
Type II cells	After 3-4 hrs by Differential centrifugation and digestion of Lung tissue with elastase as suggested by Dobbs *et al* (1986[25])

Table - II

Assessment of	methods employed
$\dot{NO}$	Acumulated $\dot{NO}$ in culture medium was measured by Greiss reaction with Na-nitrite as standard treated with nitrate reductase and NADPH.
iNOS protein expression	In cell lysate – using western blot analysis using iNOS specific antibody.
Proteins	Visualised by alkaline phosphatase
Nuclear protein	by Electrophoretic mobility shift assay using a suitable labeled probe – Protein + DNA complex separated by Polyacrylamide gel, run at 250V dried and radio autographed

$\dot{NO}$ production was more in alveolar macrophages in response to inflammatory mediator IFN-y. Laskin *et al* (1998)[4b] found that : (I) In ozone exposed alveolar macrophages $\dot{NO}$ production was double compared to when exposed to fresh air (from 11.4 to a tune of 24.2 specially in presence of LPS+IFNy), (II) This however, was more than 2.5 times in the presence of LPS+IFNy + PDTC (from 7.1 to 19.9). In type II cells (i) In the presence of LPS + IFNy ozone treated cells showed an increase to a tune of 2.72 [from 6.9 to 18.8] (ii) However, in the presence of LPS + IFNy + PDTC ozone treated cells showed an increase of 3.53 times (from 3.2 to 11.3)

Guo *et al* (1998)[26] found (using Northern blotting technique), quantified expression of iNOS in the epithelium of human airways. They found prominent iNOS gene transcription at 4.5 kb in HAEC (human airways epithelial cell) when they used 52 labeled —iNOS c-DNA probe compared to other NOS.

They also assessed NOS enzyme activity by measuring the conversion of 14 C – L – *arginine* to 14 C. L. *citrulline* in the presence of enzyme co-factors and proved that iNOS is continuously expressed in HAEC at m-RNA and protein level.

Expression of iNOS gene in HAEC in response to cytokine IFN-y, 1L–1β and TNF following results were obtained.

I. IFN-y alone induced i NOS m-RNA expression in HAEC.

II. IFN-y + 1L-4 induced more iNOS – mRNA expression than I.

III. IL-4 alone did not induce expression rather potentiates the action of IFN–y. The expressions were however, dependent on time. In certain circumstances i.e., in the presence of *cycloheximidine*, iNOS – m-RNA expression got reduced. Haque *et al* (1995)[27] found that IFN-y plays a key role in iNOS gene transcription via JAK* (Janus signal transduction pathway.

In murine *macrophages* IFN regulartory factor–1 (IRF–1) participate in iNOS activation. They further opine that IL – 4 partly mediates through $\overset{**}{STAT}-6$. Signal transduction for IFN-y is through tyrosine phosphorylation of STAT-1 Haque *et al* (1995)[27]. IL-4 mediates its effect at least in part through STAT – 6 activation Hou *et al* (1994). Guo *et al* (1994) found by experimentation that continued expression of iNOS – mRNA is both in autocrine and paracrine fashion through soluble mediators.

Inhalation of ozone (2 ppm for 3 hours) by sprague Dawley female rat cause inflammatory response (accumulation of macrophages and alveolar epithelial cell type II) at the site of injury possibly in response to inflammatory mediators lipopolysacchrides (LPS) and IFN-y which led to $\dot{NO}$ production. This is due to expression of $\dot{NO}$ synthase protein and m-RNA.

MECHANISM

Cytokine receptors are in the plasma membrane having $\propto$ & β chains. When ligand (IFN – y) binds to receptor, the signal transducing component (chain) dimerise. Inactive kinases (Tyk 1, Jac 1, Jak 2 and Jak 3) are associated with JAK. JAK are in association with chain of the receptor.

I. When ligand binds, the kinases attached to JAK become active by ligand stimulated phosphorylation. Two molecules of JAK kinases are drawn in apposition and transphosphorylate each other.
II. JAK kinases then phosphorylate a tyrosine in the distal region of β chain.
III. Active receptors then draws STAT molecule through STAT SH_2 domain.
IV. STAT molecules are phosphorylated by JAK kinases so that these too dimerise and the signaling is continued through cell surface receptors.

It has been proposed that hydrogenperoxide (TNF-$\propto$ activate Xanthine oxidase*** which generate O_2 and H_2O_2) increase the concentration of calcium (Ca^{++}). H_2O_2 induces IP_3 (inositol-1,4,5 triphosphate) which binds to receptors on endoplasmic reticulum thereby reticulum releasing Ca^{++}. The Ca^{++} stimulate phospholipase activity bringing proteolysis or possibly proteolysis is facilitated by ubiquitin which forms 26S protesosome with the target protein to be destroyed. Some scientist believe that TNF α upregulate gene expression and activities of Xanthine oxidase which generate O_2 & H_2O_2. They also believe that degradation of Ik-B is due to phosphorylated state.**

The signals are received by I_kB (inhibitory protein $_kB$). I_kB retains NF-$_kB$ (nuclear transcription factor in an inactive form. But on receipt of the signal I_kB undergoes phosphorylation* and proteolysis** as a result of which the NF-$_kB$*** (nuclear transcription factor) gets released.

After this the NF_kB is tranlocated to the nucleus and binds to regulatory motifs**** of iNOS gene. NF_kB nuclear binding activity increase in cell from ozone treated cells hence $\dot{NO}$ production in macrophages and type II cells increase. As a result of which cell injury is encountered.

CONCLUSION

Signal transduction requires signaling molecules and changes in the activity of these molecules are amplified along orchestrated signaling. A normal cell in a state oxidation reduction equilibrium (Δ Redox state) may be altered by exogenous RNS/ROS or inflammatory mediators such as TNF $\propto$/IFN-y, LPS etc. Any change or shift in the oxidation reduction equilibria modifies the regulatory protein which initiates the gene transcription Ramacle *et al,* 1995; Sen *et al,* 1996. Redox state controls the activity of NF-$_kB$ and AP_1 (Muller *et al*, 1997). Regulation of NF$_{-k}$B function via effect on I_{-k}B (inhibitors protein) is regulated by phosphorylation & proteolysis. After phosphorylation and proteolysis, NF$_{-k}$B is translocated to the nucleus where it binds to *consensus sequence* (binding sites) found in promoter region of iNOS gene. **This**

*JAK – Janus kinases

** STAT – Signal transducers and activators of transcription. Tyrosine kinase of JAK are closely attached to transmembrane receptor and get activated on ligand attachment with receptor. Activators (Enhancers) are DNA elements that facilitate or enhance gene specific proteins that regulates transcription. They facilitate binding of transcription complex to promoter region.

* Phosphorylation – Transfer of high energy phosphate.

** Proteolysis – The splitting of protein by hydrolysis of the peptide bonds with the formation of smaller polypeptides.

*** NF$_{-k}$B activily can be inhibited by *pyrrolodine* dithicarbomate (PDTC) which is an oxidant Schreck *et al* (1992[29]). Cells from ozone treated rats are less sensitive to inhibitory effect of PDTC than control.

**** Motifs – A dominant element, helps in binding of transcription factor to DNA with high affinity to the correct regions of DNA.

***** Contains FAD, molybdenum & iron, Liberates H_2O_2 which is harmful to tissues.

bindings activity is more in ozone treated cells. This leads to iNOS expression and inhancement in $\dot{N}O$ production which brings about inflammatory and pulmonary toxicity.

Laskin *et al,* 1998 hold that ozone is a toxicant and its acute inhalation results into the accumulation of macrophages at the site of injury which along with resident epithelial cells release pro-inflammatory mediators bringing toxicity. Macrophages and type II cells in response to inflammatory mediators (lipopolysacchride and TNF-α, INF-y) there was an increase in production. An explanation to this effect was due to binding of $NF_{-k}B$ to the appropriate site in iNOS gene after $I_{-k}B$ was deleted due to phosphorylation and proteolysis but Laskin *et al* (1998) did not explain the process how ozone triggered cell surface receptors via JAK kinases. This has been explained in the article (Fig.4 & 5):

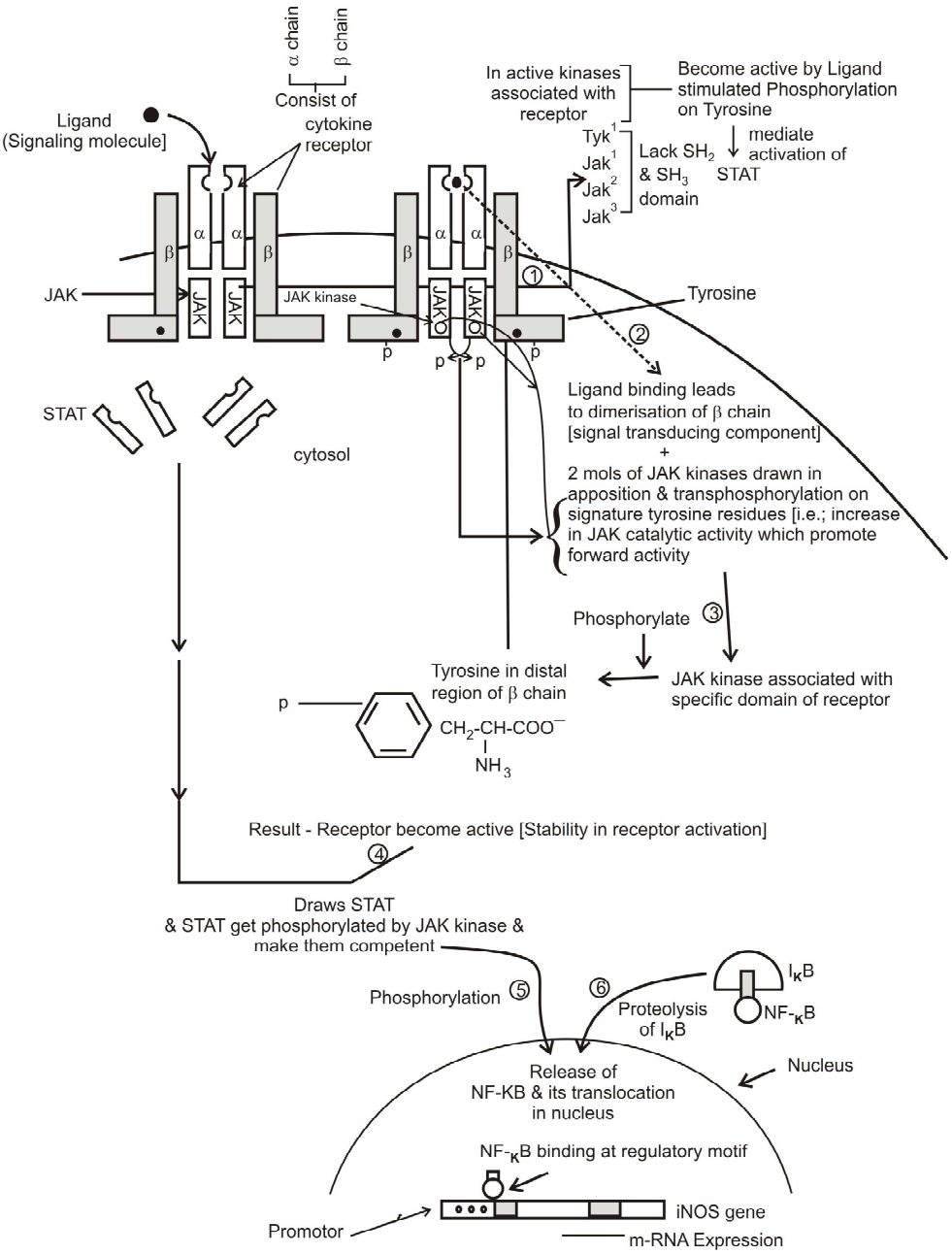

Fig. Receptor activation (JAK STAT) and m-RNA Expression of iNOS gene

Fig.4. Receptor activation (JAK STAT) and m-RNA expression of iNOS gene

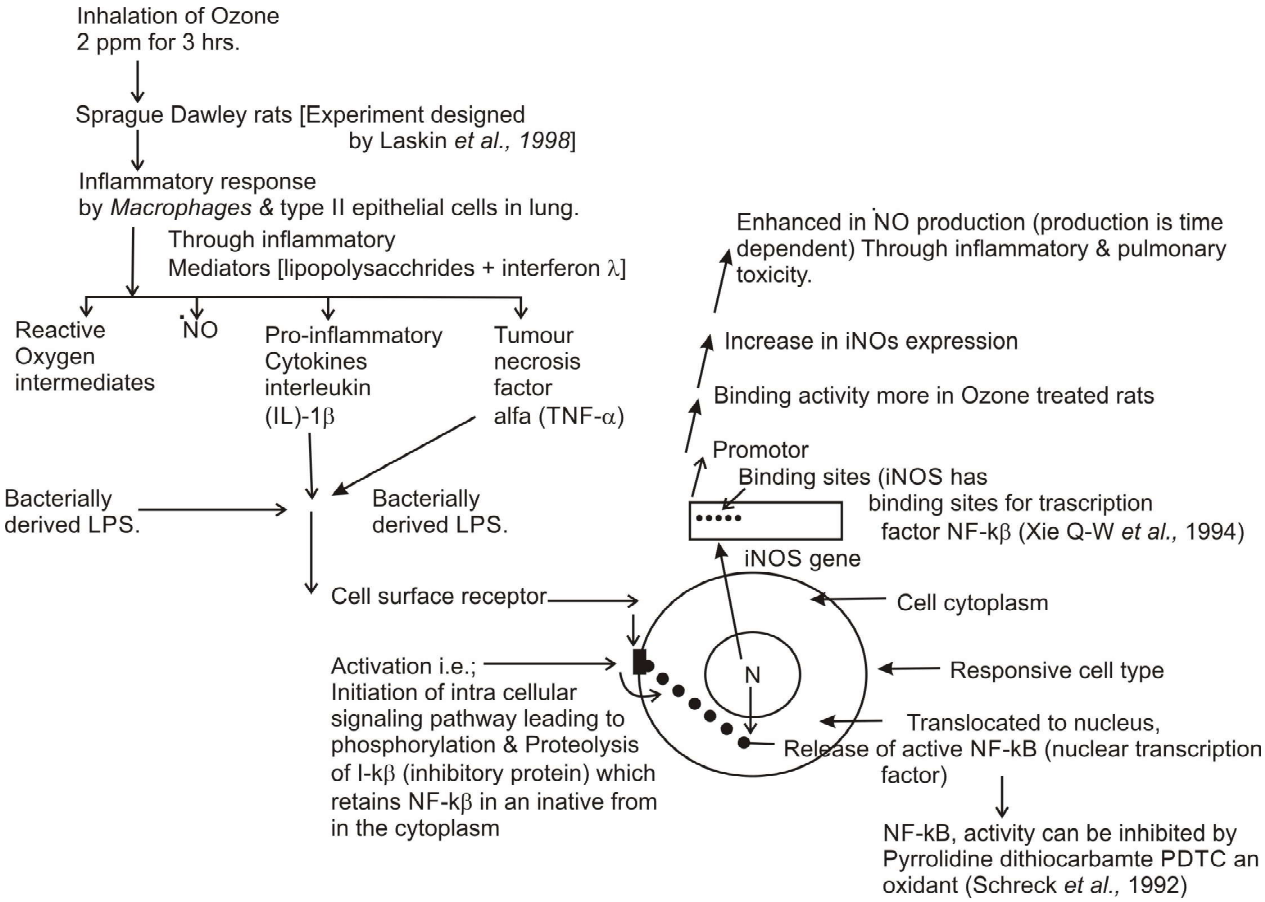

Fig.5. Diagrammatic representation of effect of Ozone inhalation on iNOS protein expression in alveolar *macrophages* & type II cells.

* Protein tyrosine phosphorylation is a tightly regulated reversible process in which forward reaction is catalysed by receptor associated Janus kinases (Jaks) & the reverse reaction by protein phosphatases (PTPS) – Heldin, 1995 & Haque & Sharma, 2006). In the absence of cytokine engagement, the receptor associated PTPS dominates over JAK, & hold the receptor in the inactive state (Fischer *et al,* 1991; Haque and Sharma, 2006). Scientist believe that inactivation of receptor associated PTPS is also an alternate means of receptor activation. Blockade of PTPS & without Cytokine binding enhances receptor activation.

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REFERENCES

1. **Dwyer, S.C., L.Legendre; P.S. Low & T.L. Leto 1996.** Plant and human neutrophil oxidative burst complexes contain immunologically related proteins. *Biochem Biophys Acta.* Vol. **1289**: 231-237.

2. **Samet J, T. Noah, R. Devlin, J. Yankaskas, K. Mckinnon, L. Dailey and M. Friedmann. 1992.** Effect of Ozone on platelet activating factor production in phorbol-differentiated HL60 cells, a human bronchial epithelial cell line (BEAS S6) and primary human bronchial epithelial cells. *Am. J. Respir. Cell Mol. Biol.* **7:** 514 – 522.

3. **Mckinnon, K.P, M.C. Madden, J.L Noah, R.B. Develin. 1993.** In vitro ozone exposure increases release of archidonic acid products from a human bronchial epithelial cell. Toxicol *Appl Pharmacol* **118**: 215-223.

4a. **Laskin, D.L. and K.J. Pendino 1995** Macrophages and inflammatory mediators in tissue injury *Annu. Rev. Pharmacol. Toxicol* **35:** 655-677.

4b. **Laskin, D.L, Sunil Vasanthi. Yan Guo, Diane E. Heck and Jeffrey D. Laskin; 1998.** Increased Nitric Oxide Synthase in Lung after Ozone inhalation is associated with activation of $NF_{-k}B$. *Env. Health Perspective.* **106(5)**: 1175-1178.

5. **Pedino K.J. D.J. Laskin, R.L. Shuler, C.J. Panjabi and D.L. Laskin. 1993.** Enhanced production of nitric oxide by rat alveolar macrophages following inhalation of

pulmonary irritant is associated with increased expression of nitric oxide synthase. *J. Immunol*; **151**: 7196-7205.

6. **Panjabi, C.J, K.J. Pindino, J.D. Laskin and D.L. Laskin. 1994;** Production of nitric oxide by rat type-II pneumocytes. Increased expression of inducible nitric oxide synthase following exposure to a pulmonary irritant *Am. J. Respir cell Mol. Biol.* **11**: 165-172.
7. **Pendino, K.J, R. Schuler, J.D. Laskin and D.L. Laskin 1994.** Enhanced production of interleukin-1, tumor necrosis factor-∝ and fibronectin by rat lung phagocytes following inhalation of pulmonary irritant. *Am. J. Respir. Cell mol. Biol*. **11**, 279-286.
8. **Lavnikova, N; S.Prokhorova; A Lakhotia; R. Gordon and D.L. Laskin. 1998.** Distinct inflammatory responses of adherent rat Lung vascular neutrophils to pulomonary irritants. *J. inflamm*. Vol **48**: 56-66.
9. **Pendino, K.J; T.M. Meidoff; D.E. Heck; J.D. Laskin and D.L. Laskin. 1995.** inhibition of macrophages with gadolonium chloride abrogates ozone induced pulmonary injury and inflammatory mediator production. *Am. J. Respir. Cell Mol. Biol.* Vol.-**13**: 125-132.
10. **Marletta, M.A. 1993.** Nitric oxide Synthase, structure and mechanism. *J. Biol. Chem*. Vol. **268:** 12231 – 12234
11. **Palmer, R.M; A.G. Ferrigl; and S. Moncada. 1987.** Nitric oxide accounts for the biological activity of endothelium derived relaxing factor. *Nature.* Vol. **327:** 524 – 526.
12. **Ignarrow, L.J; G.M. Buga; K.S. Wood; R.E. Byrns and G. Chaudhari, 1987.** Endothelial derived relaxing factor produced and released from artery and vein in nitric oxide. *Proc. Natl. Acad.* Sci U.S.A. Vol. **84**: 9265 – 9269.
13. **Furchgott, R.F. and J.V. Zawodzki. 1980.** The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature.* **288**: 373-376.
14. **Rodomski, M.W; R.M.J.K. Palmer and S.Moncada. 1987.** The anti-aggregating properties of vascular endothelium, interaction between prostacyclin and nitric oxide. *Br. J. Pharmacol.* **92:** 639-646.
15. **Snyder, S.H. 1992.** Nitric oxide, first in a new class of neurotransmitters. *Science*. **257**: 494 – 496.
16. **Breadt, D.S. and S.H. Snyder. 1989.** Nitric oxide mediates glutamate linked enhancements of cGMP level in the cerebellum. *Proc. Natl. Acad. Sci.* U.S.A. **86**: 9030 – 9033.
17. **Evans, C.H. and M. Racie Stefanovic. 1996.** Nitric oxide in arthritis. *Methods.* **10.** 38-42.
18. **Laskin, J.D; DE. Heck and D.L. Laskin 1994.** Multifunctional role of nitric oxide inflammation. TEM **5**: 377-382.
19. **Methys, K.E. and H. Bult. 1997.** Nitric oxide function is *atherosclerosis. Med. Inflamm.* **6**: 3 – 21.
20. **Beckmann, J.S. and J.P. Crow. 1993** Pathological implications of nitric oxide and peroxynitite formation (Review). *Biochem. Soc. Trans*. **21**: 330 – 334.
21. **Beckmann, J.S.; T.W. Beckmann; J.Cheng; P.A. Marshal and B.A. Freemann. 1990.** Aparant hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and Superoxide. *Proc. Natl. Acad. Sci. USA.* **87**: 1620-1624.
22. **Radi, R; J.S. Beckmann, K.M. Bush and B.A. Freeman. 1991.** Peroxynitrite induced membrane lipid peroxidation, the cytotoxic potential of super oxide and nitric oxide. *Arch. Biochem. Biophys.* **288**: 484-487.
23. **Haddad, I.Y; G. Pataki; P.Hu; C. Gillani; J.S. Beckmann and S.Metalon. 1994.** Quantitation of nitrosine levels in lung sections of patients and animals with acute lung injury. *J.Ch. Invest*. 94: 2407-2413.
24. **Lefer, D.L; R. Scalia; B. Campbell; T. Nossuli; R. Haywood; M. Salmon; J. Grayson and A.M. Lefer. 1997.** Peroxynitrite inhibits leucocyte endothelial cell interaction and protects against ischemic reperfusion injury in rats. *Lab. clin. Invest.* 99: 684-689.
25. **Dobbs, L.G; R. Gonzalez and M.C. William. 1986.** An improved method for isolating type II cells in high yield and purity. *Am. Rev. Respir. Dis.* **134**: 141-145.
26. **Guo, Y; D.E. Heck and J.D. Laskin. 1997.** Increased nitric oxide production following acute ozone inhalation is associated with activation of nuclear transcription factor NF-B. *Fundam. Appl. Toxicol.* **36**: (Supp): 8.
27. **Haque, S.J; V. Flati; A. Dab and B.R. Williams. 1995.** Role of protein tyrosine phosphatases in STAT-I alpha mediated cell signaling. *J. Biol. Chem* **270**: 25709-25714.
28. **Haque, S.J; Q.Wu; W. Kammer, K. Friedrich; K. Smith, I.M. Kerr; G.R. Stark and B.R. Williams. 1997.** Receptor associated constitutive protein tyrosine phosphatase activity control the kinase function of JAK I. Proc. Natl. *Acad. Sci.* U.S.A. **94**: 8563-8568.
29. **Shreck, R; B Meier; D.N. Mannel; W. Droge and P.A. Baeuerle. 1992.** Disthicarbamates as potent inhibitor of nuclear factor kB activation in intact cells. *J. Exp. Med.* **175**: 1181-1194.
30. **Remacle, J: M. Raes; O. Toussaint; P. Renard and G. Rao. 1995.** Low levels of reactive oxygen species as modulators of cell function. *Mutat*. Res. **316**: 103-122.
31. **Sen. C.K. L. Packer. 1996.** Antioxidant and redox regulation of gene transcription *FASEB J.* **10**: 709 – 720.
32. **Muller, J.M; R.A. Rupec and P.A. Baeucrle. 1997.** study of gene regulation of NF-kappa-B and AP_1. in response to reactive oxygen intermediates. *Methods* (Orlando) **11**: 301-312

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