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ROLE OF NITRIC OXIDE IN IMMUNITY – A REVIEW

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NO is a readily diffusible gas that has been established as a universal messenger, capable of mediating cell-cell communication throughout the body. Today, there is no simple, uniform picture of the function of NO in the immune system. Nitric oxide plays a pivotal role in cell-mediated immunity in neonates. During pregnancy NO acts as immunomodulator. NO had a role between neuroendocrine and neuroimmune systems in physiological and pathological processes. It is involved in the pathogenesis and also the control of infectious diseases, autoimmunity, chronic degenerative diseases and tumours. Thus the protective and toxic effects of NO are frequently seen in parallel. Its striking inter and intracellular signaling capacity makes it extremely difficult to predict the effect of NOS inhibitors and NO donors, which still hampers therapeutic applications. This review will collate, contrast and compare recently published literature, to provide an up-to-date overview of the substantial role that NO plays within the neonate and pregnant animal immunity.

Key word: Nitric Oxide, Immunity, Neonates, Pregnancy, Lymphocyte function

During the past two decades, nitric oxide (NO) has been recognized as one of the most versatile players in the immune system. It is involved in the pathogenesis and control of infectious diseases, tumors, autoimmune processes and chronic degenerative diseases. For the first time, Mitchell et al. (1916) reported that mammalian cells produced oxides of nitrogen. Tannenbaum and co-workers (1983) demonstrated that mammalian cells were producing nitrate (NO3-), and its production could be enhanced by endotoxin treatment. In 1985 NO formally entered the immunology scene for its role in the immune system. NO is a vasodilator, diatomic free radical, lipid soluble that reacts with a variety of molecules and mediates a large spectrum of biological effects (Nathan and Hibbs, 1991).

NO production is a feature of genuine immune-system cells (dendritic cells, NK cells, mast cells and phagocytic cells including monocytes, macrophages, microglia, Kupffer cells, eosinophils and neutrophils) as well as other cells involved in immune reactions (endothelial cells, epithelial cells, vascular smooth muscle cells, fibroblasts, keratinocytes, chondrocytes, hepatocytes, mesangial cells and Schwann cells (Bogdan, 2000). The production of NO has been thoroughly documented in cattle immune cells such as macrophages (Stuehr and Nathan, 1989), lymphocytes (Kirk et al., 1990; Reiling et al., 1996; Dixit and Parvizi, 2001) and blood leukocytes (Boulanger et al., 2001). Overproduction of NO has been observed in several inflammatory diseases. Among its many immunomodulatory properties, nitric oxide is a potent inhibitor of lymphocyte proliferation.

Several studies (Kirk et al., 1990; Reiling et al., 1996; Henson et al., 1999; Dixit and Parvizi, 2001; Boulanger et al., 2001) in bovines have shown that NO is liberated either spontaneously or after cleavage by ecto-enzymes found on T and B lymphocytes. MacMicking et al., (1997) reported that NO regulated T cell proliferation, cytokine production, apoptosis and cell signaling activity in vivo and in vitro by iNOS (inducible nitric oxide synthase) expression in immune cells. Counjun et al., (2004) reported that peripheral blood lymphocytes in bovines are capable of expressing iNOS enzyme which helps to produce NO. Bovine immune cells that are capable of expressing iNOS include macrophages (Adler et al., 1995), blood leukocytes (Boulanger et al., 2001) and lymphocytes (Dixit and Parvizi, 2001; Conjun et al., 2004).

NO biosynthesis

Palmar et al., (1988) reported that NO was synthesized from L-arginine in endothelium cells by the action of enzyme enthothelium nitric oxide synthase (eNOS) and was important for transcellular signalling. Bredt et al., (1991) reported that cytochrome P-450 like hemoproteins possess a bi-domain structure with reductase domain at the COOH terminus and an oxidative domain at the NH2 terminus. The L-arginine occurs at a heme-site in the N-terminal portion of the protein and that nitric oxide synthase may utilize a similar mechanism during generation of nitric oxide, which is paramagnetic. L-arginine reacts with both oxygen and NADPH- dependent yielding L-citrulline and NO, in a 1:1 stoichiometry (Palmer et al., 1987; Bush et al., 1992). Mammalian cells synthesize nitric oxide from the amino acid L-arginine by nitric oxide synthases through the L-arginine-nitric oxide pathway (Moncada et. al., 1993). Nathan and Xie, (1994) reported that L-arginine was essential for NO generation. L-arginine acts as a physiological nitrogen donor for nitric oxide synthase (NOS) catalyzed reactions. L-valine can be replaced for L-arginine. The competitive inhibition uptake of arginine by other naturally occurring amino acids, such as L-lysine, L-ornithine and glutamine, reduces NO synthesis (Inoue et al., 1993; Escobales et al., 2000). In most cells, arginine - the substrate for NOS is limiting and NOS can be activated by increasing arginine uptake (Dodd et al., 2000).

There are several inducing agents that activate nitric oxide synthesis within hours and inhibit DNA transcription or mRNA translation in responding cell. Inducing agents interact synergistically to enhance macrophage-mediated cytotoxicity and also interact synergistically to enhance NO release (Ding et al., 1988 and Drapier et al., 1988). A few studies in murine have indicated that cytokines (TNF-α, IFN-γ and TNF-β) enhances NO formation in vitro in peritoneal macrophages after activation with liposaccharide and in certain parasitic infections by inducing iNOS activity (Ding et al., 1990 and Silva et al., 1991). In cattle, cytokines namely IL-1, TNF-α and IFN-γ were shown to be potent modifiers of NO production in cell cultures (Adler et al., 1995). Xie et al., (1992) reported that mostly microbes or their products induce secretion of cytokines which in turn can activate nitric oxide synthase.

Nitric oxide synthases (NOS)

Nitric oxide synthases are unique among eukaryotic enzymes in being a dimeric protein in catalyzing (Marletta, 1993), the NADPH dependent reactions and requires five electron oxidation of L-arginine to generate NO and L-citrulline (Baek et al., 1993).

NOS enzymes in bovines have been characterized (Knowles et al., 1990), purified and cloned in bovine endothelium (Pollock et al., 1991) and blood leukocytes (Boulanger et al., 2001). Three isoforms depending on tissue of origin, functions and structural properties i.e. neuronal nitric oxide synthase (bNOS/nNOS/NOS1), inducible nitric oxide synthase (iNOS/NOS2) (Nathan and Hibbs, 1991; Huang, 1998) and endothelial nitric oxide synthase (eNOS/NOS3) (Bredt and Snyder, 1990) have been identified.

Constitutive nitric oxide synthases (cNOS)

Constitutive nitric oxide synthases (cNOSs) include endothelial and neuronal isoforms (eNOS and nNOS). These are found in vascular endothelium cells (Nathan and Hibbs, 1991), neuron of hippocampus (Dinerman et al., 1994), syncytiotrophoblasts (Myatt et al., 1993), platelets

(Mehta et al., 1994), neurons (Fujisawa et al., 1994), and smooth muscle (Schmidt et al., 1992). Ignarro, (1990) and Pollock et al., (1991) reported that cNOSs increased NO production in response to increase intracellular calcium levels Moncada et al., (1997) by activation of soluble guanylcyclase mechanism. Similarly Malinski and Taha, (1992) found that cNOSs once activated caused release of NO for several minutes. Stuehr, (1999) reported that nNOS and eNOS exist in the cell as preformed proteins whose activity is switched on by the elevation of intracellular Ca2+concentrations and the binding of calmodullin in response to neurotransmitters or vasoactive substances.

Inducible nitric oxide synthase (iNOS)

The inducible isoform (iNOS) is smallest isoform (molecular subunit mass of approximately 130 κDa) compared with other isoforms in bovine (Adler et al., 1995). The iNOS isoform is positively or negatively regulated by cell-cell contact (via adhesion and costimulatory molecules) in apoptotic lymphocytes (Freira-de-lima, 2000), cytokines, microbial and viral products (proteins, lipids, polysaccharides), polyamines, non-ferritin-bound iron, oxygen tension, environment pH and various antibiotics (Nathan, 1992; Bogdan, 2000). iNOS enzyme does not require calcium and calmodulin for its activation in generating NO. The enzyme activity is susceptible to profound inhibition both by corticosteroids (Di Rosa et al., 1990) and by cytokines (Ding et al., 1990) as observed in macrophages. Increased expression of iNOS by blood mononuclear cells was associated with greater production of NO in vitro in rheumatoid arthritis patients (William et al., 1996).

Physiological function of Nitric Oxide

NO mediates a variety of essential physiological function viz. vasodilation, mediation of immune defense, neurotransmission, cytoxicity and inflammation (Stuehr, 1997; Marletta et al., 1998; Geller and Billiar, 1998), modulates immune cells (Pacher et al., 2007) and smooth muscle cell proliferation (Hunt et al., 1997) Pathophysiological functions include interaction with mitochondrial systems to regulate cell respiration or cell death (Pacher et al., 2007).

Kelly, (2002) reported that nitric oxide, another proinflammatory mediator that is increased at term in maternal blood, may also contribute to vasodilation in order to facilate leukocyte trafficking. The high NO concentration around calving could exert an inhibitory effect on immune functions. These authors observed decreased immune functions after calving in first month. Kimberly et al., (2005) recorded NO profile throughout pregnancy in sheep. NO levels exhibited a biphasic pattern with the concentrations increasing over non-pregnant values on days 40-69 of gestation, returning to non-pregnant concentrations from days 70-100, and again increasing until term. These authors concluded that the pattern of the rise in peripheral NO was not directly associated with changes in vascular endothelial growth factor (VEGF) regardless of the number of fetuses present, but followed the patterns of uterine blood flow and angiogenesis of the uterus. After parturition NO metabolite concentrations in plasma were similar to non-pregnant values in ovine. Huozha et al (2010) observed comparatively higher levels of nitric oxide levels in blood plasma during gestation which significantly increased on day -7 prior to parturition ($P<0.05$) and remained high on the day of parturition The concentration started declining on day 3 postpartum. Lymphocytes contributed its share to the total pool of blood nitric oxide as was evident from nitric oxide in culture supernatant (12.5μM or less) at the end of cultured period. This suggested that buffalo lymphocytes were capable of producing nitric oxide during gestation it might be important for maintenance of pregnancy and survival of fetus.

NO and the thymus

NO has capacity to induce apoptosis (Brune et. al., 1999) because of that NO might play a role in immunology, as it is the place where T cells diversify and differentiate by positive and negative selection processes (Goldsby et al. 2003). In mouse, rat or human thymocytes, iNOS protein is absent (Tai et al., 1997; Aiello et al., 2000). By contrast, epithelial and dendritic cells in the corticomedullary junction and medulla of the thymus constitutively express iNOS, which is further upregulated after contact with self-antigens or alloantigens or with thymocytes activated by T cell–receptor (TCR) stimulation (Aiello et al., 2000; Moulian et al., 2001). TCR-activated double-positive thymocytes are highly sensitive to the killing by NO (in particular by peroxynitrite), whereas single-positive thymocytes remain viable upon exposure to NO (Fehsel et al., 1995; Brito et al., 1999). These data suggest that NO released by iNOS-positive thymic stromal cells is one of the factors mediating deletion of double-positive thymocytes. The function of eNOS expression in thymocytes is still unknown.

Role of NO in neonates

Nitric oxide plays a pivotal role in cell-mediated immunity in neonates. Research has addressed many NO-related aspects of neonatal adaptation in the time period immediately following birth (Biban, et al 2001; Colnaghi et al., 2003; Levy et al. 2005, and Christen et al., 2007). NO is involved in the killing of microorganisms and tumor cells (Nathan, 1995) and in hematopoiesis (Ouaaz, 1995). In cardiovascular adjustments and compensation, NO controls local blood flow, which ensures adequate tissue perfusion (Gow et al., 2002; Gow et al. 2004; Huang et al., 2003)

Leukocytes from calves produced unusually high concentrations of NO when compared with those produced by cows, thus constituting a possible indicator of the immaturity of the immune system of the neonatal calf (Rajaraman et al., 1998). These authors reported that leukocytes from 1-wk-old calves produced less NO and were less responsive to mitogenic stimuli than were leukocytes from older calves.

Blum et al. (1998) found very high NOx (=NO2+NO3, primarily NO3) concentration in blood plasma, saliva and urine in new-born calves before the first meal, while concentrations in their cerebrospinal fluid and in the blood plasma and milk of their dams were very low. Christen et al.,(2007) reported that neonatal cattle and in part neonates of other species have many fold higher plasma concentrations of nitrite plus nitrate than mature cows and subjects of other species, suggesting an enhanced and needed activation of the NO axis at birth. Elevated plasma concentrations of total nitrate plus nitrite, the footprint of NOS-mediated nitric oxide (NO) production from arginine (Gow et al., 2002,2004), as well as several other recent lines of evidence, suggest that the NO axis plays a critical role in the neonate's adjustments to life (Huang et al., 2003).

Role of NO on lymphocyte functions during pregnancy

Lymphocyte functions in mammals at both the peripheral and the local level changes after conception and during gestation (Jaing and Vacchio, 1998). During pregnancy (Wegmann et al., 1993) NO acts as immunomodulator produced from lymphocytes that contribute to immunomodulation. Moilanen and Vapaatalo, (1995) reported that the iNOS derived NO can synthesize cytokines and exert immunosuppressive effects by contributing to T helper -2 (Th-2) shifts and decrease T helper 1 (Th-1) cytokines during gestation in humans (Kolb and Kolb-Bachofen, 1998; Roozendaal et al., 1999) via inactivation of the zinc finger transcription factors (Berendji et al., 1999). A shift in Th-1 to Th-2 cytokines is regarded to be responsible successful pregnancy. Significantly higher secretion of NO from peripheral blood lymphocytes (PBLs) was found by Dixit and Parvizi, (2001) during different stages of gestation as compared to other physiological states (oestrous cycle). These authors concluded that PBL secreted NO for recognition and maintenance of gestation in bovine.

iNOS is the primary isoform that contributes to the majority of changes in NO production during pregnancy and labor as compared to other NOS (Nathan, (1992; Yallampalli et al., 1998; Yallampalli and Dong, 2000). Estradiol is inhibitory to iNOS expression during pregnancy and elevation in serum estrogen at term could suppress iNOS expression and thus NO production, thereby facilitating labor. These inhibitory effects of estradiol on iNOS could amplify with increasing estrogen: progesterone ratio as a result of the fall in progesterone at term (Dong et al., 1998).

Endocrine-NO interactions during pregnancy

Although a complex set of interactions regulate host defense mechanisms, communication within the neuroendocrine-immune axis is known to have major influence. During the peripartum period, a large number of reproductive, regulatory, and stress hormones are released from the anterior pituitary gland, which in turn stimulate other endocrine organs or target tissues, including those of the immune system. Glucocorticoids are known to suppress immune response and depress numbers of circulating lymphocytes. Physical and metabolic stress of pregnancy and parturition is associated with altered neuroendocrine profiles and have an impact on periparturient immune responses (Griffin, 1989; Peter and Bosu, 1987). The hormonal alterations induced by stress are responsible for the changes in cytokine concentrations because stress hormones alter the synthesis and release of the cytokines by leukocytes (Glaser et al., 1999). Secretion of immunomodulators viz NO and ACTH by peripheral blood lymphocytes during pregnancy contributes to immunomodulation for maintaining pregnancy (Dixit and Parvizi, 2001). Thus, a bidirectional communication exits between the immune and the endocrine system during pregnancy (Dixit and Parvizi, 2001).

Rettori et al., (2009) concluded that NO had a role between neuroendocrine and neuroimmune systems in physiological and pathological processes. It participates in signal transduction pathways, such as corticosterone release from the adrenal gland. Cytokine (IL-1, TNFα, IL-6, IL-2) release can stimulate adrenocorticotropic hormone release from anterior pituitary via NO.

Role of NO in lymphocyte functions (in vitro)

NO is now emerging as a potential powerful mediator of T-cell responses. It can both enhance and suppress T-cell functions and that some subsets of T-cell clones can be activated to produce NO (Liew, 1995). Among its many immunomodulatory properties, NO is a potent inhibitor of lymphocytes proliferation, cytokine production and induces apoptosis (Zamora et al., 2000; Moilanen and Vapaatalo, 1995). Albina et al., (1991) and Mills, (1991) found that NO produced from normal spleen cells can inhibit proliferation of mitogen-stimulated T cells (CD4+ T cells i.e. Th-1 cells) or enhance the suppressor function of macrophages in vitro. Others (Merryman et al., 1993; Marcinkiewicz et al., 1996) reported that the principal modulatory effect of NO was down-regulation of T cell proliferation but not cytokine production. In rodents, Taylor-Robinson et al., (1994; 1997) observed that cloned Th-1 cells expressed high levels of iNOS mRNA that produced large amounts of NO. The NO thus produced inhibited proliferation of Th-1 but not of Th-2 cells. They concluded that NO might serve as a self-regulatory molecule in preventing the over-expansion of Th-1 cells. In another study by Blesson et al., (2002) it was reported that NO produced by both cloned and naïve CD4+ T cells inhibited Th-1 cell proliferation by blocking IL-2 production more particularly and not other cytokines. Similar conclusions were drawn in earlier studies also (Taylor-Robinson et al., 1994; 1997). Bras et al., (1997) have shown that NO also exerts anti-proliferative effects on lymphocyte responses to a variety of stimuli including T cell super antigens, bacterial and parasitic infections, tumors, and allo-antigens.

Kosonen et al., (1998) reported that NO-releasing oxatriazole derivatives inhibited proliferative responses in human lymphocytes by a cGMP-independent manner. Bingisser et al., (1998) demonstrated that NO inhibited Janus tyrosine kinase-3 and signal transducer and activator of transcription-5 tyrosine phosphorylation and activation in a cyclic guanosine monophosphate-dependent manner. Miossec et al. (1997) were the first to report that caspase-3-like activity was present in non-apoptotic T cells when proliferation was stimulated with phytohemagglutinin mitogen. Caspases (cysteine aspartate protease) play an important role, not only as initiator and effector molecules in the apoptotic signaling cascade but also in T lymphocyte activation and proliferation (Kennedy et al., 1999: Alam et al., 1999). Raja et al., (2003) found that NO inhibits lymphocyte proliferation through inhibition of particularly caspase-dependent T cell proliferation by S-nitrosylation (nitrosative inactivation) of cysteine in the active site of all caspases.

CONCLUSION

In recent years NO has been found to play a much more diverse role in infection and immunity than it was initially assigned. NO has been shown to have diverse biological functions and effects within the immune system. It is an intra- and intercellular signalling molecule that can shape the immune response and sustain homeostasis. NO is a potent anti-microbial defender and has many host-protective effects that are most evident during invasion by infectious agents. It also has a possible role in thymic selection processes and the regulation of T cell differentiation. Nitric oxide plays a pivotal role in cell-mediated immunity in neonates. Leukocytes from calves produced unusually high concentrations of NO when compared with those produced by cows, thus constituting a possible indicator of the immaturity of the immune system of the neonatal calf. During pregnancy NO act as immunomodulator. NO had a role between neuroendocrine and neuroimmune systems in physiological and pathological processes. The overstimulation of NOS can assist with disease generation, inflammatory pathologies, neurodegenerative diseases and also cancer progression. In addition, nNOS and eNOS are now known to participate in important immunological processes such as apoptosis, cell adhesion, autoimmunity and perhaps antimicrobial defense. In this review, only a brief overview could be given of the relationship between nitric oxide and the immune system of neonates and pregnant animals. Further informative research into the fascinating nitric oxide molecule shall no doubt be only a short time away.

REFERENCES

1. Adler, H., Frech, B., Thony, M., Pfister, H., Peterhans, E., Jungi, T. W. (1995). Inducible nitric oxide synthase in cattle. Differential cytokine regulation of nitric oxide synthase in bovine and murine macrophages. J. Immunol. 154: 4710-4718.
2. Aiello, S., Noris, M., Piccinini, G., Tomasoni, S., Casiraghi, F., Bonazzola, S., Mister, M., Sayegh, M. H. and Remuzzi, G. (2000). Thymic dendritic cells express inducible nitric oxide synthase and generate nitric oxide in response to self- and alloantigens. J. Immunol. 164: 4649–4658.
3. Alam, A., Cohen, L. Y., Aouad, S., Sekaly, R. P. (1999). Early activation of caspases during T lymphocyte stimulation results in selective substrate cleavage in nonapoptotic cells. J. Exp. Med. 190: 1879–1890.
4. Albina, J. E., Abate, J .A., Henry, W. L. Jr. (1991). Nitric oxide production is required for murine resident peritoneal macrophages to suppress mitogen-stimulated T cell proliferation: Role of IFN-g in the induction of the nitric oxide-synthesizing pathway. J Immunol 147:144–148.
5. Baek, K. J., Thiel, B. A., Lucas, S., Stuehr, D. J. (1993). Macrophage nitric oxide synthase subunits: purification, characterization and role of prosthetic groups and substrate in regulating their association into a dimeric enzyme. J. Biol. Chem. 268:21120-21129.
6. Berendji, D., Kolb-Bachofen, V., Zipfel, P. F., Skerka, C., Carlberg, C., Kroncke, K. D. (1999). Zinc finger transcription factors as the molecular targets for nitric oxide mediated immunosuppression: inhibition of IL-2 gene expression in murine lymphocytes. Mol. Med. 11:721–730.
7. Biban, P., Zangardi, T., Baraldi, E., Dussini, N., Chiandetti, L., Zacc, F. (2001). Mixed exhaled nitric oxide and plasma nitrites and nitrates in newborn infants. Life Sci. 68: 2789–2797.
8. Bingisser, R. M., Tilbrook, P. A., Holt, P. G., Kees, U. R. (1998). Macrophage-derived nitric oxide regulates T cell activation via reversible disruption of the Jak3/STAT5 signaling pathway. J. Immunol. 160: 5729–5734.
9. Blesson, S., Thiery, J., Gaudin, C., Stancou, R., Kolb, J-P., Moreau, J-L., Theze, J., Mami-Chouaib, F., Chouaib, S. (2002). Analysis of the mechanisms of human cytotoxic T lymphocyte response inhibition by

NO. International Immunology. 14:1169-1178.

10.Blum, J. W., Husler, B., Morel, C., Egli, C. P., Kuhne, S., Rauprich, A., Hugi, D., Hammon, H., Bruckmaier, R. M., Jaggy, A., Faissler, D., Zurbriggen, A. (1998). Nitrite/nitrate concentrations in blood plasma, cerebrospinal fluid and saliva and in urinary excretions of calves: age dependency and nutritional effects. Conference of European Society of Veterinary Comparative Nutrition, Vienna, Austria, Sept. 24–28, 1998.

11.Bochsler, P. N., Mason, G. L., Olchowy, T. W. L., Yang, Z. (1996). Bacterial liposaccharide-stiumlated nitric oxide generation is unrelated to concurrent cytotoxicity of bovine alveolar macrophages. Inflmmation 20:177-189.

12.Bogdan, C. (2000). The function of nitric oxide in the immune system. In Handbook of Experimental Pharmacology. Volume: Nitric Oxide (ed. Mayer, B.) pp.443–492

Bogdan, C. (2001). Nitric oxide and the immune response. Nature Immunology 2: 907-916.

13.Boulanger, V., Bouchard, L., Zhao, X., Lacasse, P. (2001). Induction of nitric oxide production by bovine mammary epithelial cells and blood leukocytes. J. Dairy Sci. 84: 1430-1437.

14.Bras, A., Rodriguez-Borlado, L., Gonzalez-Garcia, A., Martinez, A. (1997). Nitric oxide regulates clonal expansion and activation-induced cell death triggered by Staphylococcal enterotoxin. B. Infect. Immun. 65: 4030-4037.

15.Bredt, D. S., Hwang, P. M., Glatt, C. E. (1991). Cloned and expressed nitric oxide synthase structurally resembles cytochrome P-450 reductase. Nature 351:714-718.

16.Bredt, D. S., Snyder, S. H. (1990). Isolation of nitric oxide synthase, a calmodulin-required enzyme. Proc. Natl. Acad. Sci. USA 87: 682-685.

17.Brito, C., Naviliat, M., Tiscornia, A., Vuillier, A., Gualco, G., Dighiero, G., Radi, R., Cayota, A. (1999). Peroxynitrite inhibits T lymphocyte activation and proliferation by promoting impairment of tyrosine phosphorylation and peroxinitrite-driven apoptotic death. J. Immunol. 162: 3356–3366.

18.Brune, B., von Knethen, A. & Sandau, K. B. (1999). Nitric oxide (NO): an effector of apoptosis. Cell Death Differ. 6: 969–975.

19.Bush, P. A., Gonzalez, N. E., Griscavage, J. M., Ignarro, L. J. (1992). Nitric oxide synthase from cerebellum catalyzes the formation of equimolar quantities of nitric oxide and citrulline from L-arginine. Biochem. Biophy.s Res. Commun. 185: 960-966.

20.Christen, S., Cattin, I., Knight, I., Winyard, P. G., Blum, J. W., Elsasser, T. H. (2007). Plasma S-Nitrosothiol Status in Neonatal Calves: Ontogenetic Associations with Tissue-Specific S-Nitrosylation and Nitric Oxide Synthase. Exp. Biol. Med. 232: 309-322.

21.Colnaghi, M., Condo, V., Pugni, L., Fumagalli, M., Mosca, F. (2003). Endogenous nitric oxide production in the airways of preterm and term infants. Biol. Neonate 83:113–116.

22.Denham, S., Rowland, I. J. (1992). Inhibition of the reactive proliferation of lymphocytes by activated macrophages: the role of nitric oxide. Clin. Exp. Immunol. 87: 157-162.

23.Di Rosa, M., Radomski, M., Carnuccio, R., Moncada, S. (1990). Glucocorticoids inhibit the induction of nitric oxide synthase in macrophages. Biochem. Biophys. Res. Commun. 172: 1246-1252.

24.Dinerman, J. L., Dawson, T. M., Schell, M. J., Snowman, A., Snyder, S. H. (1994). Endothelial nitric oxide synthase localized in hippocampal pyramidal cells implications for synaptic plasticity. Proc. Natl. Acad. Sci. USA 91:4214-4218.

25.Ding, A., Nathan, C. F., Graycar, J., Derynck, R., Stuehr, D. J., Srimal, S. (1990). Macrophage deactivation factor and transforming growth factors-β-1, -2, and -3 inhibit induction of macrophage nitrogen oxide synthesis by interferon-γ. J. Immunol. 145: 940 – 944.

26.Ding, A., Nathan, C. F., Stuehr, D. J. (1988). Release of reactive nitrogen intermediates and reactive oxygen intermediates from mouse peritoneal macrophages: comparison of activation cytokines and evidence for independent production. J. Immunol. 141: 2407-2412.

27.Dixit, V.D., Parvizi, N. (2001). Pregnancy Stimulates Secretion of Adrenocorticotropin and Nitric Oxide from Peripheral Bovine Lymphocytes. Biology of reproduction 64: 242-248.

28.Dodd, F., Limoges, M., Boudreau, R. T. M., Rowden, G., Murphy, P. R., Too, C.K.L. (2000). Arginine inhibits apoptosis via a NO-dependent mechanism in Nb2 lymphoma cells. Journal of Cellular Biochemistry 77: 624–634.

29.Dong, Y. L., Fang, L., Gangula, P.R.R., Yallampalli, C. (1998). Regulation of inducible nitric oxide synthase messenger ribonucleic acid expression in pregnant rat uterus. Biol. Reprod. 59: 933–940.

30.Drapier, J. C., Wietzerbin, J., Hibbs, J. B. Jr. (1988). Interferon-y and tumor necrosis factor induce the L-arginine-dependent cytotoxic effector mechanism in murine macrophages. Eur. J. Immunol. 18:1587-1592.

31.Escobales, N., Rivera-Correa, M., Altieri, P. I., Rodriguez, J. F. (2000). Relationship between NO synthesis, arginine transport, and intracellular arginine levels in vascular smooth muscle cells. Amino Acids 19: 451–468.

32.Fehsel, K., Kroncke, K-D., Meyer, K L., Huber, H., Wahn, V., Kolb-Bachofen, V. (1995). Nitric oxide induces apoptosis in mouse thymocytes. J. Immunol. 155: 2858–2865.

33.Freire-de-Lima, C. G., Nascimento, D. O., Soares, M. B., Bozza, P. T., Castro-Faria-Neto, H. C., de Mello, F. G., DosReis, G. A., Lopes, M. F. (2000). Uptake of apoptotic cells drives the growth of a pathogenic trypanosome in macrophages. Nature 403:199-203.

34.Fujisawa, K., Yagasaki, K. & Funabiki, R. (1994). Reduction of hyperlipidemia and proteinuria without growth retardation in nephritic rats by amino acids fortified low casein diets. J. Nutr. Biochem. 5: 21-27.

35.Geller, D. A., Billiar, T. R. (1998). Molecular biology of nitric oxide synthases. Cancer Metastasis Rev. 17: 7-23.

36.Glaser, R., Kiecolt-Glaser, J. K., Malarkey, W. B., Sheridan, J. F. (1998). The influence of psychological stress on the immune response to vaccines. Ann. NY Acad. Sci. 840: 649-655.

37.Goldsby, R., Kindt, T., Osborne, B. and Kuby, J. (2003). Immunology. Basingstoke: W. H. Freeman and Company.

38.Gow, A. J., Chen, Q., Hess, D. T., Day, B. J., Ischiropoulos, H., Stamler, J. S. (2002). Basal and stimulated protein S-nitrosylation in multiple cell types and tissues. J. Biol. Chem. 277: 9637–9640.

39.Gow, A. J., Farkouh, C. R., Munson, D. A., Posencheg, M. A., Ischiropoulos, H. (2004). Biological significance of nitric oxide-mediated protein modifications. Am. J. Physiol. Lung Cell. Mol. Physiol. 287: L262–L268.

40.Griffin, J. F. T., (1989). Stress and immunity: a unifying concept. Vet. Immunol. Immunopathol. 20: 263-312.

41.Henson, S. E., Nichols,T. C., Holers,V. M. & Karp, D. R. (1999). The ectoenzyme γ-glutamyl transpeptidase regulates antiproliferative effects of S-nitrosoglutathione on human T and B lymphocytes. J. Immunol. 163: 1845–1852.

42.Huang, H., Chopra, R., Verdine, G. L., Harrison, S. C. (1998). Structure of a covalently trapped catalytic complex of HIV-1 reverse transcriptase: implications for drug resistance. Science 282:1669-1675.

43.Huang, Y., Shao, X. M., Neu, J. (2003). Immunonutrients and neonates. Eur. J. Pediatr. 162: 122–128.

44.Hunt, J. S., Miller, L., Vassmer, D., Croy, B. A. (1997). Expression of inducible nitric oxide synthase gene in mouse uterine leukocytes and potential relationships with uterine function during pregnancy. Biol. Reprod. 57: 827-836.

45.Huozha, R., Sujata, P., Manju, A. (2010). Production of nitric oxide by murrah buffalo lymphocytes during gestation. Res Vet 21(1): 895-899.

Ignarro, J. J. (1990). Biosynthesis and metabolism of endothelium-derived nitric oxide. Annu. Rev. Pharmacol. Toxicol. 30: 535 – 560.

45.Inoue, Y., Bode, B. P., Beck, D. J., Li, A. P., Blend, K. I., Soube, W. W. (1993). Arginine transport in human liver. Characterization and effects of nitric oxide synthase inhibitors. Ann. Surg. 218: 350-363.

46.Jiang, S. P., Vacchio, M. S. (1998). Multiple mechanisms of peripheral T cell tolerance of fetal "allograft." J. Immunol. 160: 3086–3090.

47.Kelly, R. A., Balligand, J. L., Smith, T. N. (1996). Nitric oxide and cardiac function. Cir. Res. 79: 363-380.

48.Kennedy, N. J., Kataoka, T., Tschopp, J., Budd, R. C. (1999). Caspase activation is required for T cell proliferation. J. Exp. Med. 190: 1891– 1896.

49.Kimberly, A. V., Wilson, M. E., Li, Y., Rupnow, H. L., Phernetton, T. M., Ford, S. P., Magness, R. R. (2005). Circulating levels of nitric oxide and vascular endothelial growth factor throughout ovine pregnancy. Physiological Society 3: 552-567.

50.Kirk, S. J., Regan, M. C., Barbul, C. (1990). Cloned murine T lymphocytes synthesize a molecule with the biological characteristics of nitric oxide synthase gene. Biochem. Biophys. Res. Commun. 191: 767–774.

51.Knowles, R. G., Palacois, M., Palmar, R. M., Moncada, S. (1990). Kinetic characteristics of nitric oxide synthase from rat brain. Biochem. J. 269: 207-210.

52.Kolb, H., Kolb-Bachofen, V. (1998). Nitric oxide in autoimmune disease: cytotoxic or regulatory mediator? Immunol Today 19: 556–561.

53.Kosonen, O., Kankaaranta, H., Mari, La. Hde. Vuorinen, P., Ylitalo, P., Moilanen, E. (1998). Nitric oxide-releasing oxatriazole derivatives inhibit human lymphocyte proliferation by a cyclic GMP-independent mechanism. J. Phar. Exp. Thera. 286: 215-220.

54.Lalenti, A., Moncada, S. and Di Rosa, M. (1993). Modulation of adjuvant. arthritis by endogenous nitric oxide. Br. J. Pharmacol. 110: 701-706.

55.Lander, H. M., Sehajpal , P. K., Levine, D. M., Novogrodsky, A. (1993). Activation of human peripheral blood mononuclear cells by nitric oxide-generating compounds. J. Immunol. 150: 1509-1516.

56.Levy, M., Maurey, C., Dinh-Xuan, A. T., Vouhe, P., Israel-Biet, D. (2005). Developmental expression of vasoactive and growth factors in human lung. Role in pulmonary vascular resistance adaptation at birth. Pediatr. Res. 57: 21R–25R.

57.Liew, F. Y. (1995). Regulation of lymphocyte functions by nitric oxide. Curr. Opin. Immunol. 7: 396-399.

58.MacMicking, J., Xie, Q. W., Nathan, C. (1997). Nitric oxide and macrophage function. Annu. Rev. Immunol. 15: 323-350.

59.Malinski, T., Taha, Z. (1992). Nitric Oxide Release From a Single Cell Measured in situ by a Porphyrinic Microsensor. Nature 358: 676-678.

60.Marcinkiewicz, J., Grabowska, A. and Chain, B. M. (1996). Is there a role for nitric oxide in regulation of T cell secretion of IL-2? J. Immunol. 156: 4617-4621.

61.Marletta, M. A., (1993). Nitric oxide synthase structure and mechanism. J. Biol. Chem. 268: 12231-12234.

62.Marletta, M. A., Hurshman, A. R., Rusche, K. M. (1998). Catalysis by nitric oxide synthase. Curr. Opin. Chem. Biol. 2: 656-663.

63.Maytt, L., Brockman, D. E., Eis, A. L., Pollock, J. S. (1993). Immunohistochemical localization of nitric oxide synthase in human placenta. Placenta 14: 487-495.

64.Mehta, J. L, Lopez, L. M., Chen, L., Cox, O. E. (1994). Alterations in nitric oxide synthase activity, superoxide anion generation and platelet aggregation in systemic hypertension and effects of celiprolol. Am. J. Cardiol. 74: 901 – 905.

65.Merryma, P. F., Clancy, R. M., He, X. Y. and Abramson, S. B. (1993). Modulation of human T cell responses by nitric oxide and its derivative, S-nitrosoglutathione. Arthritis Rheum 36: 1414–1422.

66.Mills, C. D. (1991). Molecular basis of suppressor macrophages: Arginine metabolism via the nitric oxide synthase pathway. J. Immunol 146: 2719-2723.

67.Miossec, C., Dutilleul, V., Fassy, F., Diu-Hercend, A. (1997). Evidence for CPP32 activation in the absence of apoptosis during T lymphocyte stimulation. J. Biol. Chem. 272: 13459–13462.

68.Mitchell, H. H., Shonle, H. A., Grindley, H. S. (1916). The origin of nitrate in the urine. J. Bzol. Chem. 24: 461.

69.Moilanen, E., Vapaatalo, H. (1995). Nitric oxide in inflammation and immune response. Ann. Med. 27: 359-367.

70.Moilanen, E., Vuorinen, P., Kankaanranta, H., Metsa-Ketela, T., Vapaatalo, H. (1993). Inhibition by nitric oxide donors of human polymorphonuclear leukocyte functions. Br. J. Pharmacol. 109: 852-858.

71.Moncada, S., Higgs, A. and Furchgott, R. (1997). International anion of pharmacology nomenclature in nitric oxide Research. Pharmacological Reviews. 49: 137-142.

72.Moncada, S., Palmer, R. M. J., Higgs, E. A. (1993). The L-arginine nitric oxide pathway. N. Engg. J. Med. 329:2002-2012.

73.Moulian, N., Truffault, F., Gaudry-Talarmain,Y. M., Serraf,A. & Berrih-Aknin, S. (2001). In vivo and in vitro apoptosis of human thymocytes are associated with nitrotyrosine formation. Blood 97: 3521–3530.

74.Nath, J., Powledge, A. (1997). Modulation of human neutrophil inflammatory responses by nitric oxide: studies in unprimed and LPS-primed cells. J. Clin. Invest. 99:110-116.

75.Nathan, C. (1992). Nitric oxide as a secretory product of mammalian cells. FASEB J. 6: 3051–3064.

76.Nathan, C. 1995. Natural resistance and nitric oxide. Cell. 82: 873-876.

77.Nathan, C. and Xie, Q. W. (1994). Nitric oxide synthases: roles, tolls, and controls. Cell 78: 915

78.Nathan, C. F. and Hibbs, J. B., Jr. (1991). Role of nitric oxide synthesis in macrophage antimicrobial activity. Curr. Opin. Immunol. 3: 65-70.

79.Ouaaz, F. 1995. Nitric oxide in human hematopoiesis. Res. Immunol. 146: 678-681.

80.Pacher, P., Beckman, J. S., Liaudet, L. (2007). Nitric Oxide and Peroxynitrite in Health and Disease. Physiol. Rev. 87: 315-424.

81.Palmer, R. M. J., Ferrige, A. G., Moncada, S. (1987). Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 327: 524-526.

82.Palmer, R. M., Ashton, D. S. & Moncada, S. (1988). Vascular endothelial cells synthesize nitric oxide from L-arginine. Nature 333: 664-666.

83.Peter, A. T. and Bosu, W. T. K. (1987). Peripheral endocrine changes associated with retained placenta in dairy cows. Theriogenology 28:383-393.

84.Pfeiffer, S., Lass, A., Schmidt, K. & Mayer, B. (2001). Protein tyrosine nitration in cytokine-activated murine macrophages involvement of a peroxidise or nitrite pathway rather than peroxynitrite. J. Biol. Chem. 276: 34051-34058.

85.Pollock, J. S., Forstermann, U., Mitchell, J. A., Warner, T. D., Schmidt, H.H.H.W., Murad, F. (1991). Purification and characterization of particulate nitric oxide synthase from cultured and native bovine aortic endothelial cells. Proc. Natl. Acad. Sci. U.S.A. 88: 10480-10484.

86.Raja, S. M., Rosemary, A.H., Sulan, H., Amanda, W-J., Yoram, V., Richard, L. S., Timothy, R. B. (2003). Nitric oxide-mediated inhibition of caspase-dependent Tlymphocyte proliferation. J. Leu. Biology 74:403-411.

87Rajaraman, V., Nonnecke, B. J., Franklin, S. T., Hammell, D. C. and Horst, R. L. (1998). Effect of Vitamins A and E on Nitric Oxide Production by Blood Mononuclear Leukocytes from Neonatal Calves Fed Milk Replacer. J. Dairy Sci. 81: 3278-3285.

88.Reiling, N., Kroncke, R., Ulmer, A.J., Gerdes, J., Flad, H.D., Hauschildt, S. (1996). Nitric oxide synthase: expression of the endothelial, Ca2+/calmodulin-dependent isoform in human B and T lymphocytes. Eur. J. Immunol. 26: 511–516.

89.Rettori, V., Fernandez-Solari, J., Mohn, C., Zorrilla Zubilete, M. A., Cal, C. D., Prestifilippo, J. P., Laurentitis, De. A. (2009). Nitric oxide at the crossroad of immuneneuro- endocrine interactions. Ann. N Y Acad. Sci. 1153:35-47.

90.Roozendaal, R., Vellenga, E., Postma, D. S., DeMonchy, J. G., Kaufmann, H. F. (1999). Nitric oxide selectively decreases interferon gamma expression by activated human T lymphocytes via a cGMP-independent pathway. Immunology 98:393–399.

91.Schmidt, S., Wittich, R. M., Erdmann, D., Wilkes, H., Francke, W., Fortnagel, P. (1992). Biodegradation of diphenyl ether and its monohalogenated derivatives by Sphingomonas sp. strain SS3. Appl. Environ. Microbiol. 58:2744-2750.

92.Sekkai, D., Aillet, F., Israel, N. and Lepoivre, M. (1998). Inhibition of NF-kappa B and HIV-1 Long Terminal Repeat Transcriptional Activation by Inducible Nitric Oxide Synthase 2 Activity. J. Biol. Chem. 273; 3895-3900.

93.Shoker, A. S., Yang, H., Murabit, M.A., Jamil, H., Ahmed AL-Ghoul Kamal Okasha. (1997). Analysis of the in vitro effect of exogenous nitric oxide on human lymphocytes. Molecular and Cellular Biochemistry 171: 75 – 83.

94.Silva, J. S., Twardzik, D. R., Reed, S. G. (1991). Regulation of Trypanosoma cruzi infections in vitro and in vivo by transforming growth factors-β (TGF-β). J. Exp. Med. 174: 539-545.

95.Stuehr, D. J and Nathan, C. F. (1989). Nitric oxide. A macrophage product responsible for cytostasis and respiratory inhibition in tumor target cells. J. Exp. Med. 169:1543–1555.

96.Stuehr, D. J. (1997). Structure-function aspects in the nitric oxide synthases. Annu. Rev. Pharmacol. Toxicol. 37: 339–359.

97.Stuehr, D. J. (1999). Mammalian nitric oxide synthases. Biochim. Biophys. Acta 1411: 217−230.

98.Tai, X. G., Toyo-oka, K., Yamamoto, N., Yashiro, Y., Mu, J., Hamaoka, T., Fujiwara, H. (1997). Expression of an inducible type of nitric oxide (NO) synthase in the thymus and involvement of NO in deletion of TCR-stimulated double-positive thymocytes. J. Immunol. 158: 4696–4703.

99.Tannenbaum, S. R., Fett, D., Young, V. R., Land, P. D., Bruce, W. R. (1978). Nitrite and nitrate are formed by endogenous synthesis in the human intestine. Science 200:1487-1489.

100.Taylor-Robinson, A.W. (1997). Inhibition of IL-2 production by nitric oxide: A novel self-regulatory mechanism for Th-1 cell proliferation. Immunol. Cell Biol. 75:167-175.

101.Taylor-Robinson, A.W., Liew, F. Y., Severn, A., Xu, D., McSorley, S.J., Garside, P., Padron, J., Phillips, R. S. (1994). Regulation of the immune response by nitric oxide differentially produced by T helper type 1 and T helper type 2 cells. Eur. J. Immunol 24: 980-984.

102.Wegmann, T.G., Lin, H., Guilbert, L., Mosmann, T.R. (1993). Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a Th-2 Phenomenon? Immunol Today 14:353-356.

103.William, E.C., Wilkinson, W.E., Lang, T., Sanders, L., Misukonis, M.A., Gilkeson, G.S., Pisetsky, D.S., Granger, D.L., Weinberg, J.B. (1996). Increased expression of blood mononuclear cell nitric oxide synthase Type 2 in rheumatoid arthritis patients. J. Exp Med. 184:1173-1178.

104.Xie, Q.W., Cho, H.J., Calaycay, J., Mumford, R.A., Swiderek, K.M., Lee, T.D., Ding, A., Troso, T., Nathan, C., (1992). Cloning and characterization of inducible nitric oxide synthase from mouse macrophages. Science 256: 225-228.

105.Yallampalli, C. and Dong Y-L. (2000). Estradiol-17β Inhibits Nitric Oxide Synthase (NOS)-II and Stimulates NOS-III Gene Expression in the Rat Uterus. Biology of Reproduction 63: 34-41.

106.Yallampalli, C., Dong, Y.L., Gangula, P.R.R., Fang, L., (1998). Role and regulation of nitric oxide in the uterus during pregnancy and parturition. J. Soc. Gynecol. Invest. 5:58–67.

107.Zamora, R., Vodovotz, Y., Billiar, T. R. (2000). Inducible nitric oxide synthase and inflammatory diseases. Mol. Med. 6: 347–373.

