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Nitric Oxide, Nitrogen Metabolism and Inflammatory Respiratory Disease

An hypothesis

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ABSTRACT

A novel hypothesis suggesting a link between gastro-intestinal production of nitric oxide, its chemical binding in and slow release from S-nitroso proteins, and the early development of inflammatory respiratory disease is offered.

INTRODUCTORY REMARKS

Ever since the recognition of nitric oxide (NO) as the endothelium-derived relaxing factor (EDRF) (34, 18), endogenous NO production has been demonstrated in an increasing number of biological systems. Nitric oxide is involved in processes as diverse as neurotransmission, smooth muscle relaxation, bacterial killing, tumour cell lysis, and inhibition of platelet aggregation.

NO is synthesised in mammalian cells by two different types of the enzyme NO synthase (NOS). The NOS types are classified either as constitutive Ca^{2+} independent NOS (cNOS) or Ca^{2+} dependent inducible NOS (iNOS) (as reiewed by Förstermann et al. 11). Oxidation of the guanidine group of L-arginine results in the formation of NO with stoichiometric formation of L-citrulline. The enzymes are, despite the apparent simplicity of the product, large and complex haemoproteins with some similarity to cytochrome P-450 reductase, and they require multiple cofactors for their activity (47).

NO is, however, also produced by several bacterial species during the gastro-enteric denitrification of nitrite and nitrate (reviewed by Brittain et al., 8).

BACKGROUND

NO and asthmatic inflammation

The presence of nitric oxide in exhaled air and the endogenous production of NO in the lung by an NO synthase activity were first demonstrated in 1991 by Gustafsson et al. (16). A very large number of different cell types, including macrophages, neutrophils, lymphocytes, mast cells, nonadrenergic noncholinergic inhibitory neurons, fibroblasts, vascular smooth muscle cells, pulmonary endothelial and epithelial cells, have subsequently been shown to be capable of NO production in the human lung (reviewed by Gaston et al., 12).

Nitric oxide can thus be produced by most immune-competent cells, and has been implicated as an effector- and messenger-molecule in processes such as septic shock, autoimmune disease, cytotoxic defence against microorganisms and tumour cells, and modulation of cytokine production.

Asthmatic patients are known to have higher exhaled NO than healthy controls (35) and this NO production is correlated to an increase in the concentration of the inducible NO synthase in the bronchial epithelium (17).

Different cytokines are involved in asthmatic inflammation, and several of these, including interleukin-1β, tumour necrosis factor α and interferon-γ, can induce NO synthase (39). Oral administration and inhalation of anti-inflammatory corticosteroids, as well as inhalation of the NO synthase inhibitor N^G-monomethyl-L-arginine (L-NMMA), result in significant decreases in peak exhaled NO concentrations in asthmatic subjects (20, 51, 29, 21).

Cytokines in inflammatory respiratory disease

The immune system is composed of a large number of different cell types such as macrophages, mast cells, granulocytes, monocytes and lymphocytes. Lymphocytes can be subdivided into antibody producing B-lymphocytes, cytotoxic T-lymphocytes and immune-modulating T-helper lymphocytes. Based on their cytokine production profile, T-helper cells can be further subdivided into T-helper type 1 (TH1) and T-helper type 2 (TH2) cells. Interferon-γ (IFN-γ) and Interleukin 2 (IL-2) are thought to be produced exclusively by TH1 cells, whereas Interleukin 4 (IL-4) and Interleukin 5 (IL-5), for example, are produced by TH2 cells (reviewed by Mosmann and Coffman, 32).

Elevated IgE production is a major feature of atopic disease. Reciprocal roles for IL-4 and IFN-γ in the regulation of IgE production have been implicated in several in vitro studies. IL-4 is thought to be the main mediator of

the class switching to IgE production in B lymphocytes, whereas INF-γ inhibits this process.

Tang et al. (43, 44) have studied the production of IL-4 and INF-γ in peripheral blood mononuclear cells (PBMC) from atopic children. Interestingly, they have found a significantly higher IL-4 production and a lower INF-γ production in atopic children with elevated IgE production as compared with age-matched non-atopic controls. This imbalance in IL-4 and INF-γ production thus indicates a relative increase in TH2 activity and a decrease in TH1 activity in the atopic state. The other TH2 specific product IL-5, which is involved in eosinophil recruitment, has also been implicated in the pathogenesis of asthma. Doi et al. (10) have demonstrated a higher IL-5 production in PBMC from asthmatic patients as compared with non-asthmatic controls, and a normalization of IL-5 production after glucocorticoid therapy which correlated with clinical improvement of the asthmatic state.

As previously stated, these results thus imply an increase in TH2 activity with higher IL-4 and IL-5 levels and a corresponding decrease in TH1 activity in asthmatic and atopic disorders. Wheezing bronchitis is a common inflammatory respiratory disorder in early childhood which results in obstruction of the small airways (46). It is thought to be precipitated by viral infection, and is associated with the same risk factors as allergic asthma (40), a greater risk of future development of asthma (49), and the obstructive symptoms can be reduced by anti-inflammatory therapy (37).

Very little is known about cytokine profiles, IgE levels and eosinophilia associated with wheezing bronchitis. We believe, however, that some disturbances in the inflammatory response of children suffering from wheezing bronchitis are probably the same as those associated with allergic asthma.

NO and cytokine production

As previously discussed, a rapidly increasing number of studies demonstrate elevated NO production by different immune-competent cells in response to proinflammatory cytokines, and they implicate NO as one of the most important immune modulators (23).

IL-2 and INF-γ, which are produced and secreted by TH1 cells, are known to induce iNOS and subsequent NO production in macrophages (31). TH1 cells, but not TH2 cells, are themselves also capable of producing large amounts of NO in response to specific antigens or the T cell mitogen, concanavalin A (45). However, NO has been shown to inhibit both proliferation and secre-

tion of IL-2 and INF-γ from TH1 cells. Proliferation of TH2 cells and IL-4 secretion, on the other hand, are not affected by NO. Taylor-Robinson et al. (45) subsequently proposed that the nitric oxide made by TH1 cells was a self-regulatory molecule which prevented the over-expansion of TH1 cells. The inhibition of INF-γ secretion by NO, produced by macrophages, TH1 cells and other INF-γ responsive cells, may thus represent an indirect feedback mechanism which prevents excessive NO production. These results and the increased levels of NO in the exhaled air of asthmatic patients led Barnes and Liew (23) to suggest that NO, derived from airway epithelial cells, may be involved in the amplification and perpetuation of asthmatic inflammation.

Asthmatic inflammation is characterized by activation of mast cells and macrophages and infiltration of eosinophils in the airways. It was suggested that nitric oxide not only to inhibits INF-γ production and TH1 expansion but that it also causes a relative over-expansion of TH2 cells. This over-expansion of TH2 cells could explain the excessive synthesis of IL-4 (important for IgE expression) and IL-5 (plays a critical role in the recruitment of eosinophils) which is seen in PBMC from subjects suffering from atopy and asthma.

Corticosteroids are known to be effective inhibitors of iNOS (36). The exact mechanism by which steroids suppress the airway inflammation is not known, but a major mechanism of action could in fact be the suppression of iNOS expression. Steroids would thus reverse the NO induced TH1/TH2 imbalance, the relative over-expression of IL-4 and IL-5, and the perpetuation of the asthmatic inflammation by inhibiting iNOS in airway epithelial cells or macrophages.

The development of inflammatory respiratory disease

It is generally believed that the prevalence of asthma in children is increasing worldwide (50). This increase has been attributed to several different factors such as improved diagnostic capabilities regarding childhood asthma, but also air pollution and other environmental factors. Thus, development of allergic asthma and atopy is now generally considered to be a multifactorial process with an intricate interplay between genetic and environmental factors (22).

Maternal smoking during pregnancy has been shown to be associated with increased cord blood IgE levels and a subsequent development of asthma (25, 3). Paternal smoking, on the other hand, had no such effects (25).

Direct exposure to air pollutants may be a contributing factor but is probably not a major one, since the prevalence of allergies in the more polluted eastern parts of Germany has been found to be less than in the western parts (33). Environmental factors do, however, seem to influence the type of asthma that a child develops. Children born between December and February have a higher risk of becoming allergic to grass pollen, whereas children born between August and November have a higher risk of becoming sensitized to indoor allergens (15).

A large number of studies have clearly demonstrated that a genetic predisposition for the development of allergy and asthma is of major importance (19, 6). A child with no allergic parent has an approximate risk of 10% of becoming allergic. This risk is increased to 20 % with one allergic parent, and to 60% if both parents are allergic. The exact genes or loci responsible for this genetic predisposition have yet to be identified, but a linkage of high serum IgE levels to a locus on chromosome 5q31.1 has been reported (26).

There are strong indications that an imbalance in TH1 and TH2 activities, measured as INF-γ and IL-4 production, is not only present in patients with established disease, but in fact precedes clinical manifestations of atopic disease and asthma. Rinas et al. (38) have demonstrated that INF-γ production by cord blood mononuclear cells is reduced in newborn infants with a family history of atopic disease, i.e. a reduced INF-γ production during the time of first exposure to environmental antigens in the gastrointestinal and respiratory tracts. This may be of importance with respect to the development of specific allergic disorders. Furthermore, in a recent prospective study, Borres et al. (7) showed that there were significantly higher serum IL-4 levels, as early as at 3 months of age, in children who later developed atopic disease as compared with healthy controls. Serum IL-4 levels reached a peak at either 6 or 9 months in both healthy and atopic infants, and then decreased up to 18 months of age.

These results do not, of course, prove the involvement of TH1 and TH2 cells in the development of inflammatory respiratory disease. But it seems reasonable to assume that the increase in IL-4 production and the lower INF-γ levels associated with the predisposition for atopic disease, or with a presymptomatic stage of atopic disease, are the reflections of an aberration in the balance between TH1 and TH2 cells during T-helper cell development in atopic individuals.

Gastro-intestinal NO production

The intestine is the largest immunological organ in the body (2). It contains numerous immunoreactive cell types including B- and T-lymphocytes, plasma cells, macrophages, neutrophils, Paneth cells and specialized M cells. The gastro-intestinal lymphoid tissue consists of: 1) Peyer's patches, aligning the small intestine, which are the primary sites for B-cell differentiation and immunoglobulin synthesis, 2) Interepithelial lymphocytes which are predominantly cytotoxic T-cells, and 3) the immunocytes within the lamina propria consisting predominantly of cytokine producing T-helper cells. The exact function of the immune-competent cells of the gastro-intestinal tract is not known, but they are believed to serve as a barrier limiting the systemic absorption of microbes and toxins.

Nitric oxide can be produced by at least two separate mechanisms in the gastro-intestinal tract, either by iNOS in immune cells as part of an inflammatory response, or during bacterial denitrification of nitrite/nitrate. Bacterial denitrification is a process which generates nitrogen oxides (NO and N_2O) or nitrogen as final products (8). Thus far the amount of bacterially produced NO in the GI tract has received very little study. Goretski et al. (14) have reported concentrations as high as 62 nM of free NO in the aqueous phase during steady state bacterial denitrification in vitro, and Wiklund et al. (48) have reported concentrations as high as 1700 ppb (≈68nM) in the gas-phase in closed vials during cultivation of fecal specimens from SIDS (sudden infant death syndrome) victims. These numbers should be compared with the 3 nM-concentration of free nitric oxide in normal human plasma reported by Stamler et al. (41). George et al. (13) studied fecal NO concentrations in 13 breastfed children from birth to the age of 6 months. A 10-fold increase was noted (from approximately 50 to 550 ppb) predominantly from 3 to 6 months of age. There were large individual variations, however, with the lowest concentration of 15 ppb at the age of 3 days and the highest concentrations of 1440 ppb at the age of 6 months. Interestingly, this increase in NO production coincides with the maturation of the immune system, with changing patterns of cytokine production.

During recent decades, various studies have shown that the fecal microflora of children in the western world has changed from a predominantly anaerobic flora towards a more aerobic microflora, thereby eliminating the earlier difference between breastfed and bottlefed infants (9, 4, 24). The reason for this change is not clear, but the increasing use of is most likely of importance, since a large number of antibiotics, including benzylpenicillin, have been shown to lead to a pronounced suppression of the anaerobic flora (5).

Very little is known about factors that might influence the amount of NO generated in the gastrointestinal tract. Bacterial denitrification, which generates NO, N_2O and N_2, is an energy producing anaerobic process. E. Coli, as a facultative anaerobe, is not a denitrifier but can grow anaerobically by means of nitrate respiration (nitrate to nitrite). Goretski et al. (14) have shown that anaerobic bacteria will generate 10-15 times more NO when nitrite, rather than nitrate, is used as a substrate for denitrification and growth. An increasing aerobic (facultative anaerobic) gastro-intestinal microflora, grown under predominantly anaerobic conditions and generating higher faecal nitrite concentrations can thus, by means of anaerobic denitrification of nitrite, result in a substantially higher NO production as compared with the NO production expected from a strictly anaerobic flora using nitrate as a substrate for denitrification.

Nitric oxide is a very reactive molecule which has a half-life in aqueous solution on the order of seconds, resulting in plasma concentrations of free nitric oxide of approximately 3 nM. However, nitric oxide in plasma reacts with thiol groups of proteins, peptides, glutathione and cystein to form S-nitrosothiols and S-nitrosoproteins (42). These substances are far more stable, with plasma half-lives of S-nitroso proteins on the order of hours. Human plasma contains approximately 7 mM of S-nitrosothiols, a concentration 3 to 4 orders of magnitude greater than that of free NO. Free NO is, however, believed to be released from S-nitrosoprotein stores by means of mixed disulfide formation with low-molecular-weight thiols as intermediate substances. S-nitrosoproteins have been shown by Stamler et al. (41) to be bioactive as EDRF, with 82% of the S-nitrosothiols in plasma accounted for by S-nitroso-serum albumin, and Merryman et al. (30) have demonstrated that the activity of PBMC in vitro can be affected by S-nitrosoglutathion.

Nitric oxide, generated in the gastro-intestinal tract or inhaled while smoking a cigarette, can thus be stored in the plasma in large quantities and exert its effect in other parts of the body. In the form of low-molecular-weight S-nitrosothiols, it might even pass the placental barrier and affect the developing fetus.

GENERAL DISCUSSION

Allergic asthma is to a large extent a local inflammatory disorder involving the airway epithelial cells and immune-competent cells in the close vicinity to the airway epithelium. However, we would like to extend the hypothesis of Barnes and Liew. We suggest that not only is the NO derived from the airway epithelium important in amplifying and perpetuating asthmatic inflammation, but also the large quantities of NO that can be produced in the gastro-intestinal tract as well as NO in cigarette smoke, are of importance in the development of wheezing bronchitis, and atopic and asthmatic disorders.

We find it reasonable to assume that there are no qualitative differences in the way nitric oxide functions as an intra- or inter-cellular messenger in juvenile T-helper cells as compared with adult ones. That is, nitric oxide will down-regulate the expression of INF-γ, whereas the expression of IL-4 and IL-5 will be unaffected in juvenile T-helper cells even though there might be quantitative differences in the levels of cytokine expression.

It is consequently possible that bottle-feeding and the increasing use of antibiotics, resulting in disturbances of the normal anaerobic fecal microflora and higher levels of NO production during infancy, will result in a subsequent influence on the immune system.

It is also possible that the use of antibiotics during infancy will influence the development of the immune system by yet another NO/cytokine mediated mechanism. Under normal conditions the enteric anaerobic microflora will limit colonization and overgrowth of other potentially invasive microbes. However, broad-spectrum antibiotics are known to disturb this defence mechanism and lead to a colonization by potentially pathogenic Gram-negative bacilli, Gram-positive cocci and fungi (27, 28). This will in turn activate macrophages and lymphocytes in the lamina propria as well as interepithelial lymphocytes, resulting in an increase in NO production by immune cells.

Apart from a direct effect of intestinally produced NO on the TH1/TH2 balance in the lung, and especially considering the size of the gastro-intestinal tract as an "immunological organ", we find it likely that disturbances in the intestinal flora during infancy, resulting in increased NO production, will have a general immune modulating effect with a relative increase in TH2 activity and IL-4/IL-5 secretion.

An NO mediated influence on the developing immune system in utero might also explain the allergic predisposition in infants of smoking mothers. There are very high concentrations of NO in cigarette smoke (approximately 75 ppm, ref. 1). It is thus possible that the concentrations of S-

nitrosoproteins and low-molecular-weight S-nitrosothiols in the plasma of a smoking mother will generate plasma concentrations of low-molecular-weight S-nitrosothiols and free NO in the fetus that are high enough to influence the developing immune system. This could in fact explain the intriguing differences in effect of maternal vs paternal smoking during infancy.

We in no way want to suggest that NO is a prime cause of the development of allergies and asthma. However, we would like to suggest that elevated NO levels during infancy, and possibly in utero, will influence the maturation of the immune system by shifting the TH1/TH2 balance towards an increase in TH2 activity and IL-4/IL-5 production. Subsequently there will then be an indirect increase in eosinophilic activity and the development of antigen specific IgE producing B-cell clones. The immune system will thus become more prone to the development of inflammatory respiratory disorders and specific allergies, especially in individuals already genetically predisposed to the development of atopy who are exposed to large quantities of possible allergens.

We are well aware that there may be shortcomings to some aspects of this hypothesis. By and large, however, it is based on generally accepted results and observations from well performed clinical and experimental studies. For example, it has been well established that NO affects the immune system and that these effects mimic some of the abnormalities seen in asthmatic and atopic individuals. Nitric oxide has not only a local effect at the site of production, but can also exist as fairly stable S-nitrosothiol groups which exert an NO like effect in peripheral organs. There are high levels of NO in tobacco smoke, and smoking, especially maternal smoking, is a major risk factor for the development of inflammatory respiratory disorders during infancy and childhood. The intestinal microflora of children in the western world has changed during recent decades towards a more aerobic flora, and this change can be explained by nutritional factors (bottle-feeding) and the increasing use of antibiotics.

The amount of intestinally produced NO, however, must be studied more extensively. As previously discussed, there are indications that an aerobic gastro-intestinal microflora can generate higher levels of NO than an aerobic flora. Therefore possible correlations between the gastro-intestinal microflora, faecal nitrate and nitrite concentrations, NO production, serum levels of S-nitrosothiol groups and cytokine production must be elucidated.

Nevertheless, because of its possible implications for our understanding of the development of inflammatory respiratory disorders, and because of its possible importance to a large number of scientists currently investigating

quite separate aspects of allergies, nitrogen metabolism and NO production, we believe that this hypothesis should be presented and further discussed by the scientific community.

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REFERENCES

1. Alving, K., Fornhem, C. & Lundberg, J. M.: Pulmonary effects of endogenous and exogenous nitric oxide in the pig: relation to cigarette smoke inhalation. Br J Pharmacol 110: 739-746, 1993.
2. Aranow, J. S. & Fink, M. P.: Determinants of intestinal barrier failure in critical illness. British Journal Anaesth 77: 71-81, 1996.
3. Arshad, S. H., Stevens, M. & Hide, D. W.: The effect of genetic and environmental factors on the prevalence of allergic disorders at the age of two years. Clin Exp Allergy 23: 504-511, 1993.
4. Beerens, H., Romond, C. & Neut, C.: Influence of breast-feeding on the bifid flora of the newborn intestine. Am J Clin Nutr 33: 2434-2439, 1980.
5. Bennet, R., Eriksson, M., Nord, C.-E. & Zetterström, R.: Fecal bacterial microflora of newborn infants during intensive care management and treatment with five antibiotic regimens. Pediatr Infect Dis 5: 533-539, 1986.
6. Björksten, B.: Risk factors in early childhood for the development of atopic disease. Allergy 49: 400-407, 1994.
7. Borres, M. P., Einarsson, R. & Björksten, B.: Serum levels of interleukin-4, soluble CD23 and INFγ in relation to the development of allergic disease during the first 18 months of life. Clin Exp Allergy 25: 543-548, 1995.
8. Brittain, T., Blackmore, R., Greenwood, C. & Thomson, A. J.: Bacterial nitrite-reducing enzymes. Eur J Biochem 209: 793-802, 1992.
9. Bullen, C. L., Tearle, P. V. & Willis, A. T.: Bifidobacteria in the intestinal tract of infants: An in vitro study. 9: 225-233, 1976.
10. Doi, S., Gemou-Engesaeth, V., Kay, A. B. & Corrigan, C. J.: Polymerase chain reaction quantification of cytokine messenger RNA expression in peripheral blood mononuclear cells of patients with acute exacerbations of asthma: effect of glucocorticoid therapy. Clin Exp Allergy 24: 854-867, 1994.
11. Förstermann, U., Schmidt, H. H. H. W., Pollock, J. S., Sheng, H., Mitchell, J. A , Warner, T. D., Nakane, M. & Murad, F.: Isoforms of nitric oxide synthase: characterization and purification from different cell types. Biochem Pharmacol 42: 1849-1857, 1991.

12. Gaston, B., Drazen, J. M., Loscalzo, J. & Stamler, J. S.: The biology of nitrogen oxides in the airways. Am J Respir Crit Care Med 149: 538-551, 1994.

13. George, M., Nord, K.-E., Ronquist, G., Hedenstierna, G. & Wiklund, L.: Faecal microflora and urease activity during the first six months of infancy. Ups J Med 101:233-250, 1996.

14. Goretski, J., Zafiriou, O. C. & Hollocher, T. C.: Steady-state nitric oxide concentrations during denitrification. J Biol Chem 265: 11535-11538, 1990.

15. de Groot, H., Stapel, S. O. & Aalberse, R. C.: Statistical analysis of IgE antibodies to the common inhalant allergens in 44496 sera. Ann Allergy 65: 97-104, 1990.

16. Gustafsson, L. E., Leone, A. M., Persson, M. G., Wiklund, N. P. & Moncada, S.: Endogenous nitric oxide is present in the exhaled air of rabbits, guinea pigs and humans. Biochem Biophys Res Commun 181: 852-857, 1991.

17. Hamid, Q., Springhall, D. R., Riveros-Moreno, V., Chanez, P., Howarth, P., Redington, A., Bousquet, J., Godard, P., Holgate, S. & Polak, J. M.: Induction of nitric oxide synthase in asthma. Lancet 342: 1510-1513, 1993.

18. Ignarro, L. J., Buga, G. M., Wood, K. S., Byrns, R. E. & Chaudhuri, G.: Endothelium derived relaxing factor produced and released from artery and vein is nitric oxide. Proc Natl Acad Sci USA 84: 9265-9269, 1987.

19. Kaufman, H. S. & Frick, O. L.: The development of allergy in infants of allergic parents: a prospective study concerning the role of heredity. Ann Allergy 37: 410-415, 1976.

20. Kharitonov, S. A., Yates, D., Robbins, R. A., Logan-Sinclair, R., Shinebourne, E. A. & Barnes, P. J.: Increased nitric oxide in exhaled air of asthmatic patients. Lancet 343: 133-135, 1994.

21. Kharitonov, S. A., Yates, D. H. & Barnes, P. J.: Inhaled glucocorticoids decrease nitric oxide in exhaled air of asthmatic patients. Am J Respir Crit Care Med 153: 454-457, 1996.

22. Koning, H., Baert, M. R. M., Oranje, A. P., Savelkoul, H. F. J. & Neijens, H. J.: Development of immune functions related to allergic mechanisms in young children. Pediatr Res 40: 363-375, 1996.

23. Barnes, P. J. & Liew, F. Y.: Nitric oxide and asthmatic inflammation. Imm Today 16: 128-130, 1995.

24. Lundequist, B., Nord, C. E. & Winberg, J.: The composition of the faecal microflora in breastfed and bottlefed infants from birth to eight weeks. Acta Paediatr Scand 74: 45-51, 1985.

25. Magnusson, C. G. M.: Maternal smoking influences cord serum IgE and IgD levels and increases the risk for subsequent infant allery. J Allergy Clin Immunol 4: 182-186, 1986.

26. Marsh, D. G., Neely, J. D., Breazeale, D. R., Ghosh, B., Freidhoff, L. R., Ehrlich-Kautzky, E., Schou, C., Krishnaswams, G. & Beaty, T. H.: Linkage

analysis of IL-4 and other chromosome 5q31.1 markers and total serum immunoglobulin E concentrations. Science 264: 1152-1156, 1994.

27. Marshall, J. C., Christou, N. V., Horn, R. & Meakins, J. L.: The microbiology of multiple organ failure: the proximal gastrointestinal tract as an occult reservoir of pathogens. Arch Surgery 123: 309-315, 1988.

28. Marshall, J. C., Christou, N. V. & Meakins, J. L.: The gastrointestinal tract. The "undrained abscess" of multiple organ failure. Annals Surgery 218: 111-119, 1993.

29. Massaro, A. F., Gaston, B., Kita, D., Fanta, C., Stamler, J. S. & Drazen, J. M.: Expired nitric oxide levels during treatment of acute asthma. Am J Respir Crit Care Med 152: 800-803, 1995.

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

31. Moncada, S., Marletta, M. A., Hibbs, J. B. & Higgs, E. A. (Eds.): The biology of nitric oxide. Portland Press, Colchester, 1993.

32. Mosmann, T. R. & Coffman, R. L.: TH1 and TH2 cells: Different patterns of lymphokine secretion lead to different functional properties. Ann Rev Immunol 7: 145-173, 1989.

33. von Mutius, E., Fritzsch, C., Weiland, S. K., Roll, G. & Magnussen, H.: Prevalence of asthma and allergic disorders among children in united Germany: a descriptive comparison. BMJ 305: 1395-1399, 1992.

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

35. Persson, M. G., Zetterström, O., Agreniusm, V., Ihre, E. & Gustafsson, L. E.: Single-breath nitric oxide measurements in asthmatic patients and smoking. Lancet 343: 146-147, 1994.

36. Radomski, M. W., Palmer, R. M. J. & Moncada, S.: Glucocorticoids inhibit the expression of an inducible, but not the constitutive, nitric oxide synthase in vascular endothelial cells. Proc Natl Acad Sci USA 87: 10043-10049, 1990.

37. Reijonen, T., Korppi, M., Kuikka, L. & Remes, K.: Anti-inflammatory therapy reduces wheezing after bronchiolitis. Arch Pediatr Adolesc Med 150: 512-517, 1996.

38. Rinas, U., Horneff, G. & Wahn, V.: Interferon gamma production by cord blood mononuclear cells is reduced in newborns with a family history of atopic disease and is independent from cord blood IgE levels. Paediatr Allergy Immunol 4: 60-64, 1993.

39. Robbins, R. A., Barnes, P. J., Springall, D. R., Warren, J. B., Kwon, O. J., Buttery, L. D. K., Wilson, A. J., Geller, D. A. & Polak, J. M.: Expression of inducible nitric oxide in human bronchial epithelial cells. Biochem Biophys Res Commun 203: 209-218, 1994.

40. Rylander, E., Pershagen, G., Eriksson, M. & Nordvall, L.: Parental smoking and other risk factors for wheezing bronchitis in children. Eur J Epidemiol 9: 517-526, 1993.

41. Stamler, J. S., Simon, D. I., Osborne, J. A., Mullins, M. E., Jaraki, O., Michel, T., Singel, D. J. & Loscalzo, J.: S-nitrosylation of proteins with nitric oxide synthesis and characterization of biologically active compounds. Proc Natl Acad Sci 89: 444-448, 1992.

42. Stamler, J. S., Jaraki, O., Osborne, J., Simon, D. I., Keaney, J., Vita, J., Singel, D., Valeri, C. R. & Loscalzo, J.: Nitric oxide circulates in mammalian plasma primarily as an S-nitroso adduct of serum albumin. Proc Natl Acad Sci 89: 7674-7677, 1992.

43. Tang, M., Kemp, A. & Varigos, G.: IL-4 and interferon-gamma production in children with atopic disease. Clin Exp Immunol 92: 120-124, 1993.

44. Tang, M. L. K., Coleman, J. & Kemp, A. S.: Interleukin-4 and intarferon-gamma production in atopic and non-atopic children with asthma. Clin Exp Allergy 25: 515-521, 1995.

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

46. Welliver, J. R. & Welliver, C.: Bronchiolitis. Pediatr Rev 14: 134-139, 1993.

47. White, K. A. & Marletta, M. A.: Nitric oxide synthase is a cytochrome P-450 type hemoprotein. Biochemistry 31: 6627-6630, 1992.

48. Wiklund, L., George, M., Nord, C. E., Ronquist, G. & Saldeen, T.: Sudden infant death syndrome and nitrogen metabolism; further development of an hypothesis. Submitted, 1997.

49. Wilson, N. M.: Wheezy bronchitis revisited. Arch Dis Child 64: 1194-1199, 1989.

50. Woolcock, A. J., Peat, J. K. & Trevillion, L. M.: Changing prevalence of allergies worldwide. In: Johansson SGO (ed) Progression allergy and clinical immunology, Vol III. pp 167-171, Hografe & Huber, Gottinger, Germany, 1995.

51. Yates, D. H., Kharitonov, S. A., Robbins, R. A., Thomas, P. S. & Barnes, P. J.: Effect of a nitric oxide synthase inhibitor and a gluco-corticosteroid on exhaled nitric oxide. Am J Respir Crit Care Med 152: 892-896, 1995.

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