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SCIENTIFIC REVIEW

NITRIC OXIDE AND INFLAMMATORY JOINT DISEASES

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SUMMARY

Nitric oxide (NO) is synthesized from L-arginine by the NO synthases. At present, mainly three NO synthase isoenzyme groups are differentiated: two constitutive NO synthases, responsible for homeostatic cardiovascular and neuronal functions of NO, and an inducible NO synthase. After induction by certain cytokines or endotoxin, this latter isoform produces large quantities of NO with cyto- and bacteriotoxic effects. High amounts of NO, synthesized systemically and intra-articularly, play an important role in inflammatory joint diseases, as shown in animal models of arthritis and in patients with rheumatoid arthritis or spondyloarthropathies. In experimental arthritis, administration of NO synthase inhibitors profoundly reduced disease activity. In humans, beneficial effects of NO synthesis inhibition are inferred from indirect evidence: glucocorticoids, inhibiting induction of the inducible NO synthase, reduce enhanced NO synthesis and disease activity. Thus, selective inhibition of the pathologically enhanced NO synthesis emerges as a new experimental therapeutic approach in the treatment of inflammatory joint diseases.

KEY WORDS: Nitric oxide, Metabolism, Inflammatory joint disease, Treatment, Isoenzymes, Nitric oxide synthase.

IT had already been suggested in 1916 that mammals synthesize nitrate, but the source remained unclear [1]. Seven decades later, several independent streams of research led to the discovery of nitric oxide (NO) as a secretory product of mammals (for a review, see [2]). (1) Research on the effector mechanisms of cytotoxicity of macrophages led to the finding that cytotoxicity was linked to nitrite/nitrate production and that L-arginine is necessary for this effect [3, 4]. (2) Investigations on acetylcholine-dependent vasodilation led to the discovery of the endothelium-derived relaxing factor (EDRF), synthesized by vascular endothelial cells [5] and acting by stimulation of guanylyl cyclase [6]. This enzyme had been previously shown to be activated by NO [7]. (3) Studies of nitrosamine generation and metabolism demonstrated that rats and humans excrete more nitrate than they ingest [8, 9]. Inflammatory stimuli like endotoxin enhanced nitrate and nitrite production accompanied by nitrosamine formation, which stems from a reactive precursor originating from oxidation of L-arginine [10, 11].

These lines of investigation converged in 1987/88 on the identification of NO as the active principle of EDRF [12, 13] and as an important effector molecule of immune responses [14]. Furthermore, its action as a neurotransmitter was recognized [15]. Subsequently, the NO pathway became a main field of interest of many investigators and NO was elected 'molecule of the year' in 1992 by *Science* [16].

NO SYNTHESIS

NO originates from oxidation of a guanidino nitrogen of L-arginine [17, 18]. This reaction is catalysed by the NO synthase (NOS). So far, two constitutive and one inducible NO synthase have been distinguished. The constitutive NO synthases (NOS I in nerve cells [19–21], NOS III in endothelial cells [22–24]) regulate homeostasis of blood flow, platelet function and signal transduction in the central and peripheral nervous systems. The NO generation of these NO synthases is controlled by enzyme activation or inhibition [25]. In contrast to the constitutive NO synthases, the inducible NO synthase is expressed only 'on demand' and is controlled by the rate of enzyme turnover [25, 26–29]. Nearly all body cells are able to express this enzyme [2, 25, 30], particularly macrophages/monocytes [14, 31], neutrophils [32, 33] and hepatocytes [34, 35]. Expression of the inducible NO synthase is induced by endotoxin and cytokines, especially tumour necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), interleukin (IL)-1 and IL-2 [36–40]. Other cytokines, such as transforming growth factor beta, and IL-4, -8, -10 and -13 suppress *de novo* synthesis of the enzyme [41–45]; also glucocorticoids inhibit inducible NO synthase expression [46, 47].

Another difference between the constitutive and inducible enzymes is due to their Ca^{2+} dependence. All NO synthases depend on NADPH, flavin nucleotides, tetrahydrobiopterin and calmodulin as cofactors [48–51]. Calmodulin is extremely tightly bound to the inducible isoenzyme [52], thus inducible NO synthase activity is Ca^{2+} independent, whereas the constitutive enzymes need Ca^{2+} for association of calmodulin [25].

However, in human hepatocytes, an inducible NO synthase partly dependent on Ca^{2+} was found [53]. Also, a Ca^{2+}-dependent inducible NO synthase exists in rabbit and human chondrocytes, which is not suppressible by glucocorticoids [54, 55]. Thus, the inducible NO synthase is showing differences depending on the cell type. In fact, the amino acid sequences are different, corresponding by only 80% [53].

The constitutive NO synthases in nerve cells and endothelial cells, and the inducible NO synthase, are

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different proteins, their amino acid sequences showing only 52–58% homology. Within mammalian species, the amino acid sequences of each NO synthase isoenzyme are highly conserved, showing homologies of >90% (NOS I and NOS III) and >80% (NOS II), respectively [25].

These molecular biological findings are of great importance. The differences between constitutive and inducible NO synthases make it possible to design highly selective inhibitors of the NO isoenzymes. At present, such inhibitors with sufficient selectivity are not available. The NO synthase inhibitors most often used experimentally are: N^G-monomethyl-L-arginine (L-NMMA), N^G-nitro-L-arginine-methyl-ester (L-NAME) and aminoguanidine. The L-arginine analogues L-NMMA and L-NAME are unselective inhibitors, whereas aminoguanidine, with 10- to 100-fold higher potency, preferably inhibits the inducible NO synthase [56].

MOLECULAR ACTIONS OF NO

The molecular modes of action of NO are summarized in Table I. NO exerts its cardiovascular effects by activation of the soluble guanylyl cyclase [6, 7, 57]. The subsequently synthesized cyclic guanosine monophosphate (cGMP) is an effective vasodilator and inhibitor of platelet aggregation [58, 59]. Essential for the cyto- and bacteriotoxic actions of NO are inactivation of mitochondrial enzymes [60], damage to nucleic acids [61] and formation of peroxynitrite, which is generated by the reaction of NO with the superoxide anion [62]. Decomposition of peroxynitrite yields hydroxyl radicals, which are strong oxidants, contributing to killing of microorganisms, but also to tissue damage [62]. Furthermore, iron ions, liberated by binding of NO to ferritin, augment the formation of radicals [63].

NO also affects the activity of cyclo-oxygenase, a key enzyme of prostanoid synthesis. Similar to the NO synthases, a constitutive cyclo-oxygenase with homeostatic functions and an inducible cyclo-oxygenase exist; the latter is responsible for the synthesis of pro-inflammatory prostanoids [64]. *In vivo*, the inducible forms of inducible NO synthase and cyclo-oxygenase are expressed following stimulation by the same cytokines. Increased NO formation enhances the activity of both cyclo-oxygenase isoenzymes, resulting in pro-inflammatory prostanoid synthesis [65]. The *in vitro* findings are divergent, resulting in increased or decreased activity of cyclo-oxygenase [66] depending on NO concentration and the presence of other radicals.

NO METABOLISM AND ANALYSIS

In biological fluids, NO has a half-life of only a few seconds, because it is readily oxidized to nitrite and subsequently to nitrate, which are excreted in the urine [67]. NO can react with thiol groups of amino acids and proteins to *S*-nitroso compounds, which are of great interest, because they exert higher vasodilatory potency than NO itself and have at least similar anti-aggregatory potency [68]. However, the mechanisms of formation and physiological role of these *S*-nitroso compounds from NO are still under discussion [69].

Because of its short half-life, NO itself is difficult to measure directly *in vivo*. Thus, the NO metabolites nitrite and nitrate serve as index parameters of NO production. Moreover, serum nitrate and nitrite reflect NO synthesis occurring at the time of sampling [67, 70, 71]; nitrate excretion in urine over 24 h is an index of daily NO synthesis [72, 73]. A prerequisite for the use of these metabolites to determine NO production is the absence of excessive or variable dietary nitrite/nitrate ingestion. It had already been shown before the discovery of the NO pathway that people on diets normal or low in nitrate excrete more nitrate in the urine than they ingested in their diet [9]. Only 40–60% of excreted nitrate can be accounted for by dietary

TABLE I
Molecular actions of NO

Target	Effect	Result	Ref. no.
1. Iron-containing proteins			
Soluble guanylyl cyclase	Enhanced enzyme activity	cGMP production enhanced, causing vasodilatation, inhibition of platelet aggregation, neurotransmission	15, 57, 58, 59
Enzymes of respiratory chain and citric acid cycle	Inhibition of mitochondrial respiration	Cytotoxicity	60
Cyclo-oxygenases	Enhanced enzyme activity	Enhanced prostanoid synthesis	65, 66
Haemoglobin (Hb)	Formation of Met-Hb	Met-Hb: inactivation of NO by oxidation	67
	Formation of *S*-nitroso-Hb	*S*-nitroso-Hb: endogenous NO donator	68, 69
Ribonucleotide reductase	Inhibition of enzyme activity	Mutagenesis, cytotoxicity	61, 168
ADP-ribosyltransferase	Enhanced enzyme activity	Inhibition of glycolysis, cytotoxicity	166, 167
Ferritin	Liberation of iron	Promotion of radical formation	63
2. Superoxide anion	Formation of peroxynitrite	Inactivation of superoxide, but formation of hydroxyl radicals. Cytotoxicity	62
3. Nucleic acids	DNA deamination, oxidation, strand breaks, cross-links	Mutagenesis, cytotoxicity	61
4. Thiol group-containing amino acids, peptides, proteins	Formation of high- and low-molecular-weight *S*-nitroso compounds	Endogenous NO donators?	68, 69

intake [9, 74]. Therefore, in the absence of excess dietary nitrate intake, the major source of urinary nitrate is endogenously synthesized NO.

Several methods for nitrite/nitrate measurement in biological fluids have been established (for a review, see [30, 75]). The Griess reaction [76] and its modification by Green *et al.* [77] is the simplest and most frequently used assay. However, the gold standard for assessment of nitrite and/or nitrate is gas chromatography–mass spectrometry with the use of ^{15}N-labelled nitrate and nitrite, respectively, as internal standards [75, 78]. For further information about the cellular and enzymatic origin of NO, the enzyme activity, expression of NO synthase antigen and mRNA levels can be determined *ex vivo* and *in vitro*.

NO AND INFLAMMATION

In inflammatory reactions, pro-inflammatory cytokines lead to expression of the inducible NO synthase in monocytes/macrophages, neutrophil granulocytes and many other cells; in the case of bacterial infection, endotoxin is another strong inducer of expression [2, 14, 25, 30–40, 79]. In consequence, large amounts of NO are synthesized, exceeding the physiological NO production by up to 1000-fold.

The generated NO acts through various molecular targets (Table I) toxic to bacteria, fungi, protozoa, helminths and malignant cells [80–83]. Thus, high amounts of NO produced by the inducible NO synthase perform important functions for host defence, but could also damage intact tissue [84] and the NO-producing cells themselves [85].

Before discussing the evidence for an involvement of NO in the pathomechanism of chronic inflammatory, non-infectious diseases, it must be stressed that low amounts of NO, as produced by the constitutive NO synthases, are protective for inflammatory tissue damage. NO suppresses T-cell proliferation, T-cell activity, chemotaxis of monocytes, adhesion and migration of neutrophil granulocytes [86–90]. NO inhibits the synthesis of IL-6, prostaglandin E_2 and thromboxane in macrophages [91], as well as liberation of platelet activating factor and histamine from mast cells [92, 93]. Furthermore, the vasodilating and anti-aggregatory effects of NO contribute to its protective properties via maintenance of perfusion. Accordingly, inhibition of these anti-inflammatory and protective properties of NO can result in inflammation and promotion of tissue injury: ileitis can be induced by treatment of healthy guinea pigs with the unselective NO synthase inhibitor L-NAME [94]. In contrast, aminoguanidine, which preferentially inhibits the inducible NO synthase, yields no inflammation, because the inducible NO synthase is expressed in healthy mammals in negligible amounts and has apparently no currently recognized physiological importance [94]. Another example is experimental liver cell necrosis induced by injection of killed *Corynebacterium parvum* and endotoxin in the mouse. In this animal model of acute hepatic damage, treatment with L-NMMA enhances liver injury by occlusion of small vessels [95, 96].

To summarize: depending on its concentration and origin, NO possesses pro- as well as anti-inflammatory effects [56, 79, 97]. These properties of NO must be considered in interpreting the results of studies and have led NO to be called a 'double-edged sword' [56] or 'Jekyll and Hyde' [98].

NO IN INFLAMMATORY JOINT DISEASES

The *in vivo* synthesis of NO and its pathophysiological implications are well documented in animal models of arthritis as well as in human inflammatory joint diseases.

Animal experimental studies

Animal experiments convincingly show involvement of NO in the development and maintenance of arthritic conditions [99–110]. In rats suffering from adjuvant arthritis, we have demonstrated that the urinary nitrate excretion was increased by >3-fold as compared to healthy controls [99]. Urinary cGMP excretion was not increased in these rats, pointing to an activation of the inducible form of the NO synthase, because urinary nitrate and cGMP excretion are influenced in parallel when the constitutive NO synthase is activated [111, 112]. Treatment of adjuvant arthritic rats with L-NAME reduced urinary nitrate excretion and disease activity, as assessed by paw volume and arthritis score, to the same extent as dexamethasone (Figs 1 and 2) [100]. In the course of adjuvant arthritis, urinary nitrate excretion increased as the arthritis progressed. The development of adjuvant arthritis could be suppressed by L-NAME or L-NMMA in a dose-dependent manner. L-Arginine abrogated the suppression by L-NAME and L-NMA, respectively [101, 102]. Ialenti *et al.* [103] demonstrated elevated nitrite generation by peritoneal macrophages collected from rats with adjuvant arthritis. Nitrite generation and the severity of arthritis were exacerbated by L-arginine, the source for NO production, and suppressed by L-NAME.

In rats with arthritis, induced by injection of streptococcal cell wall fragments, McCartney-Francis *et al.* [104] found elevated NO production by synovial tissue of inflamed joints and by polymorphonuclear cells. Administration of L-NMMA profoundly reduced NO production by synovial tissue, synovial inflammation and tissue damage.

Also in the third animal model of systemic inflammatory joint disease—collagen-induced arthritis in rats—NO production, measured as urinary nitrate excretion, and expression of the inducible NO synthase in lymphatic tissues and joints increased in parallel to the development of arthritis [109]. Pathophysiological differences between collagen-induced arthritis and adjuvant arthritis were reflected by later onset and lower maximal values of urinary nitrate excretion and expression of the inducible NO synthase as compared to adjuvant arthritis in rats of the same strain and age [109].

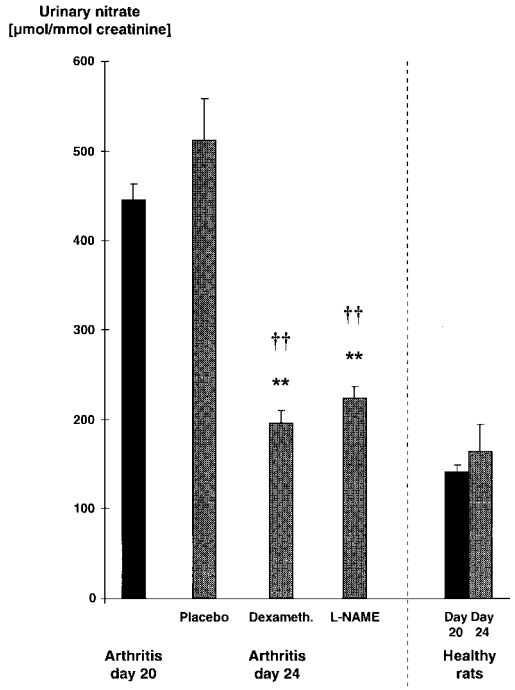

FIG. 1.—Nitrate excretion in 24 h urine of adjuvant arthritic rats before and after treatment (day 20–24 after induction of arthritis) with dexamethasone (0.5 mg p.o./kg body weight/day; $n = 12$), L-NAME (20 mg p.o./kg body weight b.i.d; $n = 12$) or placebo ($n = 12$). Healthy rats of the same strain and age acted as controls ($n = 12$). $^{**}P < 0.001$ for comparison of day 24 *vs* 20 (two-tailed paired *t*-test). $^{\dagger\dagger}P < 0.001$ for comparison of dexamethasone and L-NAME, respectively, at day 24 *vs* placebo at day 24 (Scheffé's *F*-test). Data are from [100].

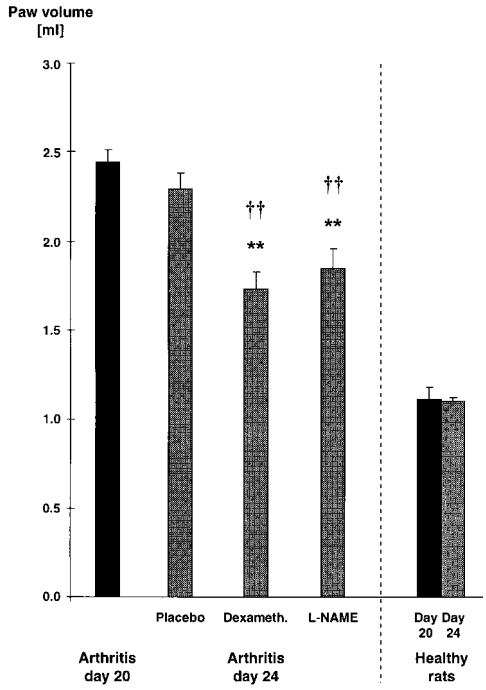

FIG. 2.—Paw volume of adjuvant arthritic rats before and after treatment (day 20–24 after induction of arthritis) with dexamethasone (0.5 mg p.o./kg body weight/day; $n = 12$), L-NAME (20 mg p.o./kg body weight b.i.d; $n = 12$) or placebo ($n = 12$). Healthy rats of the same strain and age acted as controls ($n = 12$). $^{**}P < 0.001$ for comparison of day 24 *vs* 20 (two-tailed paired *t*-test). $^{\dagger\dagger}P < 0.001$ for comparison of dexamethasone and L-NAME, respectively, at day 24 *vs* placebo at day 24 (Scheffé's *F*-test). Data are from [100].

Human studies

In man, several studies confirm the animal experiment results that NO is not only a marker, but also a pro-inflammatory mediator of arthritis.

Farrell and co-workers [71] found increased serum concentrations of nitrite, indicating enhanced NO production, in serum and synovial fluid of patients with rheumatoid arthritis (RA) and osteoarthritis (OA). In the latter group, serum nitrite levels were half as high as in patients with RA, but still 1.5-fold higher than in age- and sex-matched healthy controls. In both groups, nitrite concentrations in synovial fluid were higher than in the corresponding serum samples, indicating NO production in the inflamed joint.

In patients with an acute flare of RA, we determined urinary nitrate excretion before and after treatment with prednisolone (Fig. 3) [113]. Before the start of prednisolone therapy, the urinary nitrate excretion of the patients was 2.7-fold higher than that in healthy volunteers. The urinary nitrate excretion was decreased significantly by therapy with prednisolone, when C-reactive protein concentrations were normalized and clinical disease activity was reduced considerably. Despite this decrease, the urinary nitrate excretion was still 2-fold higher in patients than in the control group, probably due to local production of NO in the arthritic joints.

Subsequently, these findings were confirmed and extended by two other studies in RA: Grabowski *et al.* [114] could also demonstrate a 3-fold higher urinary nitrate excretion in patients with RA who were not taking oral corticosteroids; unfortunately, no data on disease activity were given. Ueki *et al.* [115] analysed nitrite concentrations in serum and synovia of patients with RA and OA. The patients with RA had 8-fold higher serum nitrite concentrations than OA patients and healthy controls. In both patient groups, synovial nitrite concentrations were higher than serum nitrite concentrations, again indicating intra-articular NO production. Interestingly, in this study serum nitrite concentrations correlated significantly with clinical and laboratory parameters of disease activity as well as with serum TNF-α and IL-6 levels [115].

A similar correlation between NO and systemic disease activity was previously found in patients with spondyloarthropathies as defined by the classification criteria of the European Spondylarthropathy Study Group [116]. The serum nitrate concentrations of spondyloarthropathy patients with systemic inflammatory activity were >2-fold increased in comparison to the patients without systemic active disease and healthy controls (Fig. 4) [117]. The serum nitrate concentrations of spondyloarthropathy patients correlated closely with C-reactive protein and erythrocyte

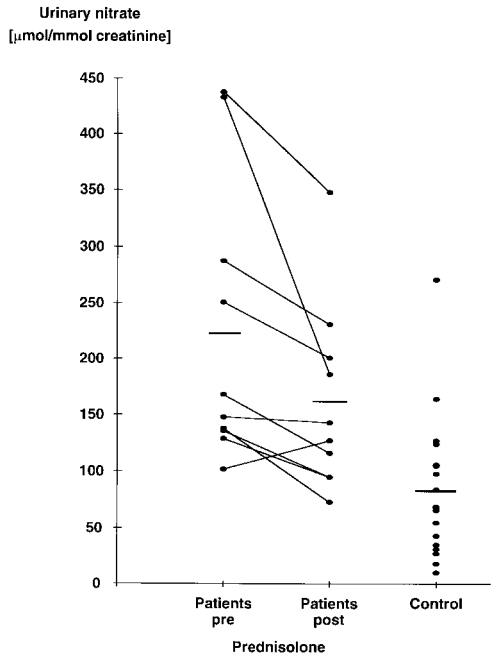

FIG. 3.—Urinary nitrate excretion of patients with rheumatoid arthritis ($n = 10$) before and after the start of prednisolone 0.5 mg/kg body weight. Healthy volunteers ($n = 18$) who were comparable in age and gender acted as controls. $P < 0.001$ for patients pre-prednisolone *vs* control. $P < 0.05$ for patients pre-prednisolone *vs* post-prednisolone and for patients post-prednisolone *vs* control. Reproduced from [113] with the kind permission of the *Annals of the Rheumatic Diseases*.

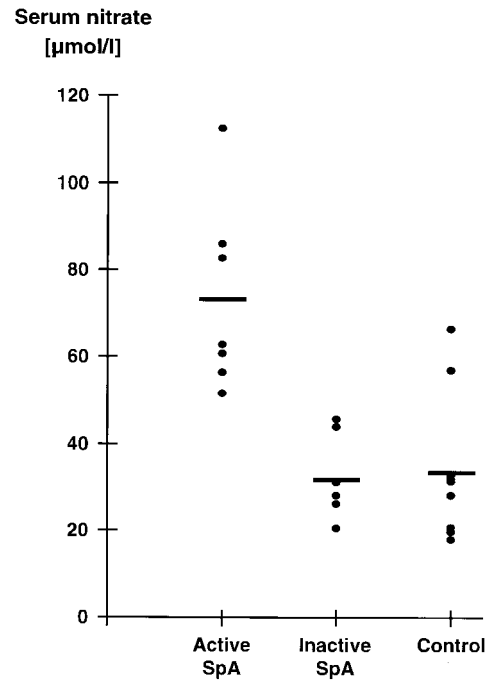

FIG. 4.—Serum nitrate concentrations in patients with active/inactive ($n = 7$ each) spondyloarthropathy (SpA) and healthy volunteers ($n = 10$). In Scheffé's *F*-test, active SpA *vs* inactive SpA and control, respectively, $P < 0.001$. The figure is from [117].

sedimentation rate (Fig. 5). In a recent double-blind, placebo-controlled randomized study, we found almost doubled serum nitrate concentrations in patients with active ankylosing spondylitis as compared to healthy controls. Interestingly, a 6 week treatment with non-steroidal anti-inflammatory drugs reduced clinical disease activity, but serum nitrate and C-reactive protein concentrations remained unchanged (D. O. Stichtenoth and J. C. Frölich, unpublished data).

Further evidence for an involvement of the NO pathway in inflammatory joint diseases is given by the measurement of elevated nitrotyrosine concentrations in serum and synovial fluid from patients with RA, but not in control subjects [118]. Nitrotyrosine is formed by reaction of peroxynitrite with tyrosine and is an index parameter of NO-dependent oxidative damage [119].

NO may also be important for the pathogenesis of systemic lupus erythematosus. MRL-lpr/lpr mice, the animal model of systemic lupus erythematosus, excrete more nitrite/nitrate in urine than control strains and this urinary nitrite/nitrate excretion corresponds to the onset of glomerulonephritis and arthritis. Treatment with L-NMMA reduced urinary nitrite/nitrate excretion and prevented or significantly decreased clinical, laboratory and histological parameters of disease activity [120]. Furthermore, serum nitrite concentrations in lupus pregnancies with and without pre-eclampsia were increased, particularly in those with active disease, as compared to healthy pregnancies or pre-eclampsia [121].

Cellular origin and actions of NO in arthritis

As mentioned above, nearly all mammalian cells can express the inducible NO synthase after stimulation by cytokines, mainly IL-1, TFN-α and IFN-γ, which are known to be enhanced in inflammatory joint diseases [122, 123]. In humans, the following extra- and intra-articular sources of inflammatory NO production were identified: synovial fibroblasts [124], synoviocytes [125], endothelial cells [125], monocytes/macrophages in blood stream and synovial membrane [124, 126], osteoblasts [127, 128] and chondrocytes [125, 129–131], particularly superficial chondrocytes [132]. In patients with active RA, blood mononuclear cells had increased NO synthase activity due to expression of the inducible isoenzyme; the NO synthase activity correlated with the tender and swollen joint count [126]. The intra-articular NO stems predominantly from synovial fibroblasts, as shown by McInnes *et al.* [124] in primary synovial cultures from RA patients. Interestingly, only a limited number of synovial macrophages, which are able to express high levels of inducible NO synthase, were found to be activated in the inflamed synovium.

Macrophages of rodents are a rich source of NO and can be stimulated easily by endotoxin, TNF-α or IFN-γ [2–4, 14, 25, 40]. Surprisingly, there has been a long-standing controversy on NO production by human macrophages. Many attempts with the use of the above-mentioned standard procedures of

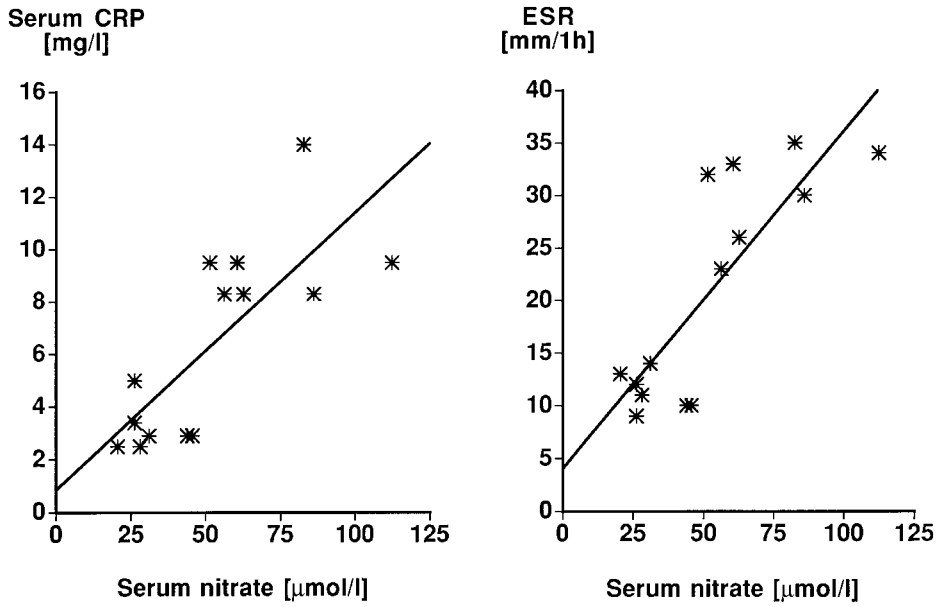

FIG. 5.—Correlations of serum nitrate *vs* C-reactive protein (CRP)/erythrocyte sedimentation rate (ESR) in patients with SpA ($n = 14$). Coefficient of correlation for serum nitrate *vs* CRP: $r = 0.77$; $P < 0.001$. Coefficient of correlation for serum nitrate *vs* ESR: $r = 0.83$; $P < 0.001$. CRP values were highly correlated to ESR: $r = 0.94$; $P < 0.001$. The figure is from [117].

stimulation failed completely or NO production was undetectable despite expression of inducible NO synthase [31, 133]. Even when stimulation was successful, e.g. by co-incubation with tumour cells [134], infection with microorganisms [135] or cross-linking of CD69 receptors [136], human macrophages did not produce NO in amounts as high as rodent macrophages. This highlights the significant role of other cells for NO production in human inflammatory diseases.

Physiological NO production inhibits bone resorption by osteoclasts [137] and NO may have acute, but not chronic, protective effects in IL-1β-induced matrix breakdown of bovine cartilage cultures [138]. However, the high amounts of NO produced by inflamed synovium lead to enhanced bone resorption [139, 140] and diminished bone proliferation [127, 141], decreased proteoglycan synthesis [142], activate metalloproteases [143] and induce chondrocyte apoptosis [144]. All of these effects contribute to joint damage, thus NO must be considered as an important effector molecule of disease progression.

OTHER CHRONIC INFLAMMATORY DISEASES

The role of NO as a pro-inflammatory mediator is proven for other chronic inflammatory diseases, corroborating the findings given above. In different animal models of chronic inflammatory bowel diseases, an increased NO production by the inducible NO synthase was found. Cellular sources of this NO production were mucosal neutrophils in the acute phase, and monocytes/macrophages and lymphocytes in the chronic phase. Inhibition of NO synthesis by L-NAME or aminoguanidine reduced both NO synthesis and disease activity [94, 145].

Ex vivo experiments with colonic mucosa biopsy specimens of patients with an acute flare-up of ulcerative colitis or Crohn's disease demonstrate enhanced activity of NO synthase in the inflamed mucosa [146, 147]. This activity was Ca^{2+} independent, suggesting expression of the inducible NO synthase. Corresponding to our study in RA [113] are the results of Sasajima *et al.* [148]: patients with acute flare-up of ulcerative colitis have a 2-fold increased urinary nitrite/nitrate excretion as compared to healthy controls. After treatment with hydrocortisone and sulphasalazine, when the disease became inactive, urinary nitrite/nitrate excretion normalized with the exception of one patient who failed to respond to treatment.

An involvement of NO in the pathomechanism of disease is also postulated and more or less well described in asthma [149, 150], allograft rejection [151, 152], streptozotocin-induced diabetes mellitus [153], autoimmune encephalomyelitis [154] and glomerulonephritis [155, 156].

DRUG EFFECTS ON NO SYNTHESIS AND THERAPEUTIC IMPLICATIONS

The selective inhibition of enhanced NO synthesis is a new, so far exclusively experimental, therapeutic strategy in the treatment of chronic inflammatory, non-infectious diseases.

Some established drugs for the therapy of these diseases inhibit activity or expression, respectively, of the inducible NO synthase, which may contribute to their anti-inflammatory effects: glucocorticoids inhibit expression of the inducible NO synthase, but have no effects on the activity of both inducible and constitutive NO synthases [46, 47]. The mechanism of action is complex and includes inhibition of transcription and translation, as well as reduced enzyme stability [157]. As a result, the pathologically enhanced NO synthesis

and disease activity in RA are reduced by glucocorticoids [113].

Cyclosporin derivatives inhibit NO synthase expression. This could be explained by their actions on IL secretion and by direct effects on gene transcription [158]. Similar effects on the expression of inducible NO synthase are described for non-steroidal anti-inflammatory drugs [159, 160]. However, the mechanism and clinical implications of these findings remain unclear. In addition, salicylates are scavengers of NO [161]; recently, 5-aminosalicylic acid was found to reduce both NO production and disease activity in adjuvant arthritic rats [162]. Equally well described, but not explained, is the inhibition of NO production of endotoxin-stimulated mouse macrophages by auranofin [163]. Methotrexate blocks the synthesis of tetrahydrobiopterin, one of the essential cofactors of the NO synthases. This decreased tetrahydrobiopterin availability may be of therapeutic relevance because it is rate limiting, particularly for the inducible NO synthase with its high enzyme activity [56, 164].

Specific and selective inhibition of the inducible NO synthase is so far possible only in animal experiments to some extent. For use in humans, only highly selective and non-toxic substances are suitable, since the pro-inflammatory NO production by the inducible NO synthesis, but not the homeostatic NO synthesis by the constitutive enzymes, must be inhibited. The latter inhibition would lead to vasoconstriction [165] and platelet aggregation, both of which would augment the inflammatory tissue damage [94, 95].

A number of substances for selective inhibition of pathological NO overproduction are now under development. Besides selective inhibition of inducible NO synthase activity, several other targets of pharmacological intervention have emerged: inhibition of enzyme transcription and translation, cofactor and substrate supply.

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