Nitric Oxide as an Inflammatory Mediator in Autoimmune MRL-\textit{lpr}/\textit{lpr} Mice

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Nitric oxide (NO) may exhibit proinflammatory features. NO synthase type 2 (NOS2) is overexpressed and NO overproduced in rodent models of induced inflammation. Blockage of NOS production by administration of NOS inhibitors prevents or reduces various types of induced inflammation in mice and rats. We have shown that autoimmune MRL-\textit{lpr}/\textit{lpr} mice overexpress NOS2 and overproduce NO in an age-dependent fashion that parallels expression of arthritis, glomerulonephritis, and vasculitis. Blocking NO production by oral administration of the NOS inhibitor N\textsuperscript{\,-,}monomethyl-L-arginine reduced the arthritis, glomerulonephritis, and vasculitis, but it did not modify serum anti-DNA antibody levels or glomerular deposition of immune complexes. When mice with genetically disrupted NOS2 were backcrossed to MRL-\textit{lpr}/\textit{lpr} mice, the resultant (-/-) mice expressed no NOS2 and produced no NO, the wild-type (+/+). mice overexpressed NOS2 and overproduced NO (in comparison to normal, control mice), and the heterozygous (+/-) mice expressed and produced intermediate levels. Nephritis and arthritis in the (-/-) mice were comparable to that in MRL-\textit{lpr}/\textit{lpr}-/mice, but vasculitis was markedly decreased. Levels of anti-DNA antibodies were comparable in all mice, but IgG rheumatoid factor production was markedly reduced in the (-/-) mice. These results of studies in MRL-\textit{lpr}/\textit{lpr} mice with genetically disrupted NOS2 highlight the heterogeneity and complexity of the role of NOS2 and NO in inflammation.

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Overview

Inflammatory joint tissue in rheumatoid arthritis (RA) is characterized by infiltration and accumulation of mononuclear phagocytes, lymphocytes, and plasma cells, proliferation of synovial cells, and expression of proinflammatory cytokines (1–3). Although some cytokines are undetectable or are present in only low levels in rheumatoid synovia, synovial macrophages and fibroblasts in this disease are a good source of cytokines such as interferon (IFN)-\textgreek{a}, interleukin (IL)-1, tumor necrosis factor (TNF)-\textgreek{a}, IL-6, IL-8, and granulocyte macrophage–colony-stimulating factor (4–7). In addition to these protein mediators, arachidonic acid metabolites, reactive oxygen species ([ROS], superoxide anion radical \textgreek{O}_2^-\textsuperscript{--}, hydrogen peroxide, and hydroxyl radical), and reactive nitrogen species (nitric oxide [*NO], and related molecules such as peroxynitrite [OONO\textsuperscript{--}]) likely contribute to the pathology. The combined actions of these mediators (along with certain inherent and induced anti-inflammatory mediators) contribute to the eventual pathology—accumulation of inflammatory cells, modification of synovial vascular cells, proliferation of synovial fibroblasts, disruption of the general synovial architecture, and destruction of cartilage and bone. NO, \textgreek{O}_2^-\textsuperscript{--}, and OONO\textsuperscript{--} appear to be central to the inflammatory process. Antioxidant enzymes such as catalase, superoxide dismutase (SOD), and glutathione peroxidase may be critical as endogenous defenses against inflammation. Various cells in the joint may participate in the inflammation. These include mononuclear phagocytes and chondrocytes.

\textbf{NO and Nitric Oxide Synthase}

The simple gas NO has multiple important physiologic and pathologic functions (8–10 for reviews). These include roles in (to mention only a few) host resistance to tumors and microbes, regulation of blood pressure and vascular tone, neurotransmission, learning, and neurotoxicity, carcinogenesis, and control of cellular growth and differentiation (11,12). In the presence of oxygen, NO rapidly (seconds) is converted to nitrite and nitrate, substances that are generally not bioactive (13 for review). NO binds with high affinity to iron in heme groups of proteins such as hemoglobin (Hb), myoglobin (Mb), and guanylyl cyclase; Hb and Mb are very effective quenchers of NO action. NO also reacts with \textgreek{O}_2^-\textsuperscript{--}, and SOD prolongs NO life by eliminating \textgreek{O}_2^-\textsuperscript{--}. On reacting with \textgreek{O}_2^-\textsuperscript{--}, NO may form OONO\textsuperscript{--}, a very toxic/reactive molecule that may be the most important effector toxic molecule when one thinks of NO toxicity in oxygenated systems.

There are three forms of the enzyme nitric oxide synthase (NOS) encoded by three different genes. Neural NOS (nNOS or NOS1) and endothelial cell NOS (\textgreek{e}NOS or NOS3) are constitutive enzymes, demonstrating low-level, constant transcription of mRNA. The enzymatic actions of NOS1 and NOS3 are modulated by regulation of cytoplasmic calcium levels, with agents inducing increases in calcium (e.g., calcium ionophores, ligands such as acetylcholine, or mechanical stress), with subsequent binding to calmodulin and activation of..
the enzyme. Inducible NOS (iNOS or NOS2) can be regulated at multiple levels, but induction of mRNA transcription by agents such as cytokines or lipopolysaccharide (LPS) appears to be of major importance [(14) for review]. The activity of NOS2 is generally thought to be calcium independent. Although NOS2 was described initially in mononuclear phagocytes, it also is found in synoviocytes, chondrocytes, smooth muscle cells, hepatocytes, and others (8,9,15–17).

Regulation of NOS2 can occur at multiple steps (14), including mRNA transcription, stability, and translation. At the protein level, NOS may be regulated by calmodulin binding, dimer formation (the functional enzyme exists as a dimer), substrate (L-arginine) depletion, substrate recycling (L-citrulline to L-arginine), tetrahydrobiopterin availability, end product inhibition (‘NO interaction with NOS heme), phosphorylation, and subcellular localization. Important NOS cofactors include FAD, FMN, NADPH, tetrahydrobiopterin, and calmodulin–calcium. For NOS2, calmodulin is tightly bound to protein, making it relatively resistant to inhibition by calcium chelators. Activities of NOS can be influenced by tetrahydrobiopterin levels, and cytokines/LPS can enhance tetrahydrobiopterin production (18,19). Heme is a critical component of NOS; ‘NO can act as a feedback inhibitor of NOS activity by binding to the iron in heme (20,21).

**Human Mononuclear Phagocyte NOS2 Expression and ‘NO Production**

Several researchers have shown high-level ‘NO production by murine macrophages, but many have difficulty showing that human monocytes or macrophages produce ‘NO in vitro [(22) for review]. Because of this, some investigators have questioned the roles of mononuclear phagocyte NOS2 and ‘NO in human disease. However, human hepatocytes, chondrocytes, and human DLD-1 colon tumor cells can express NOS2 mRNA and produce high levels of ‘NO after treatment with cytokines and LPS (15,16,23–25). Some have shown human monocyte ‘NO production, but levels have been low when compared to murine mononuclear phagocytes [(22,26–28) for review]. In a study of normal human monocytes and peritoneal macrophages, we showed that these cells can produce NOS2 mRNA, protein, and ‘NO, but levels were much less than that of mouse macrophages (29). We examined a large array of culture conditions and cytokines. IFN-γ and LPS showed only slight activity. Human alveolar macrophages have NOS2 antigen (30,31), and patients with tuberculosis have increased numbers of alveolar macrophages that express NOS2 antigen (32). Humans can be induced to make high levels of ‘NO in vivo via an NOS mechanism (33), but the cells responsible for ‘NO production in vivo are not known. We have recently noted that children with mild malaria overproduce ‘NO and have mononuclear cells that express NOS2 antigen (34). We have also noted that RA patients have overexpression of NOS2 antigen in blood mononuclear cells, and that their monocytes have enhanced ‘NO production in vitro and enhanced responsiveness to treatment with IFN-γ and LPS (35). Thus, human mononuclear phagocytes can express NOS2 and generate ‘NO, and human NOS2 expression and ‘NO generation correlate with the severity of certain diseases, including the autoimmune disease RA.

**‘NO and Inflammation**

‘NO has many actions appropriate for a proinflammatory agent. It is made by numerous cell types in sites of inflammation, and it increases blood flow and vascular permeability. ‘NO has cell/tissue destructive abilities, and it can induce cyclooxygenase (COX), cause pain, destroy certain protease inhibitors, and enhance production of IL-1 and TNF, and NADPH oxidase activity in myeloid cells (11,36).

‘NO production may result from the actions of several substances including cytokines, immune complexes, and bacterial products (Figure 1). Because O$_2^\cdot$ may interact with ‘NO to produce OONO$^-$, coincident production of ‘NO and O$_2^\cdot$ sets the stage for a severe inflammatory state. In the joint, several cell types may produce ‘NO—macrophages, chondrocytes, endothelial cells, and possibly others.

Macrophages and chondrocytes are the most likely contributors. Human articular chondrocytes produce relatively high levels of ‘NO, and cytokines can augment this production (15,37,38). Synovial fibroblasts in patients with arthritis also express NOS2 and produce ‘NO (39). Because several pathways might lead to increased ‘NO production, blocking only one pathway (e.g., blocking an IFN alone) might not fully blunt ‘NO production, since alternate paths could compensate (Figure 1).

Researchers have noted that in addition to proinflammatory effects, ‘NO may also be anti-inflammatory. The double-edged sword phenomenon in ‘NO biology thus applies to inflammation as well as to other areas in ‘NO biology (8,40). ‘NO modifies adhesiveness and chemotaxis of polymorphonuclear neutrophils and monocytes (41,42), inhibits platelet aggregation and secretion (43), and inhibits cell proliferation [including lymphocytes (8)].

Arachidonic acid metabolites play important roles in inflammation, and COX inhibitors are drugs useful in the management of inflammatory disease (44). There is significant cross-talk between ‘NO and COX. Eicosanoids can reduce NOS2 expression and ‘NO production (45–47), and ‘NO modulates prostaglandin E$_2$ formation (48). Stimuli that enhance NOS2 and ‘NO formation also may induce COX2 expression, but the time course for induction differs (49–51). Arginine analogues such as NG-monomethyl-L-arginine (NMMA) may be anti-inflammatory by inhibiting both COX2 and NOS2 (52). Furthermore, aspirin, in high doses, inhibits both cyclooxygenase and NOS2 (53).

‘NO is important in animal models of arthritis that mimic human RA. These include adjuvant arthritis, collagen-induced arthritis, and spontaneous arthritis in MRL mice [(36,38,54) for reviews]. Likewise, ‘NO participates in the
pathogenesis of spontaneous myositis in the SJL mouse (55). Mice with genetically disrupted transforming growth factor β1 (TGF-β) have severe multifocal inflammation, and they die after only 2 to 3 weeks of life. These mice have overexpression of ROS2 and overproduction of 'NO (56). The overproduction of NO is inhibited by treatment with NMMA in vivo. The absence of TGF-β, an inhibitor of ROS2 transcription and translation (57), appears to cause a systemic lethal inflammatory condition that results from 'NO overproduction.

'NO may be increased in synovial fluid and serum of patients with RA (58–60). Kaur and Halliwell (61) showed increased levels of nitrotyrosine (a product resulting from OONO− action) in serum and synovial fluid from arthritis patients (61). Sakurai et al. (62) found that synovia from patients with RA and osteoarthritis (OA) produce 'NO in vitro, and express ROS2 mRNA (reverse transcriptase–polymerase chain reaction) and protein (immunoblot and immunohistochemistry). The ROS2 was associated primarily with CD14+ cells (mononuclear phagocytes). Other investigators have also found that synovial fibroblasts (as well as macrophages) from patients with RA and OA produced 'NO and expressed ROS2 (39). Ueki and co-workers (63) showed that RA patients have higher serum 'NO than do osteoarthritis (OA) patients, that RA synovial fluid 'NO was much higher than serum 'NO, and that serum 'NO levels correlated significantly with clinical parameters of disease activity (duration of morning stiffness, number of swollen and tender joints, and serum levels of C-reactive protein, TNF, and IL-6). We had earlier noted comparable associations (35).

The MRL-lpr/lpr Model of Autoimmunity

These mice develop a disease that resembles human systemic lupus erythematosus and RA (64). They have autoantibodies, rheumatoid factor, arthritis, nephritis, and vasculitis, and they die prematurely from disease. They have lymphadenopathy and splenomegaly, and a defect in apoptosis caused by a mutated fas gene (an insert of the early transposable element resulting in aberrant splicing and premature termination of transcription) (65). Fas protein is a membrane protein that, after ligation with an antibody or with its ligand FasL, causes cell death by apoptosis. δδH−δδl−−l−− mice are similar to MRL-lpr/lpr mice in that they have generalized lymphadenopathy and a defect in apoptosis. The gld defect is a defect in the fasL gene, that encodes the ligand for Fas. Mice of both strains have increased numbers of CD4+, CD8+, and CD3+ cells. These cells are polyclonal and nonmalignant. Fas is essential for the activation-induced death of mature T cells in the periphery but not in the thymus. In MRL-lpr/lpr mice, failure of apoptosis results in persistence of autoreactive T cells that help autoreactive B cells that are not eliminated. Genetic factors in addition to lpr are also important in determination of disease. MRL mice without the fas abnormality still develop an autoimmune disorder (albeit less severe). Our work showed while enhanced ROS2 expression and 'NO production are critical for disease development, disease likely does not result from an abnormality in the ROS2 gene per se (66). Because these mice have high serum and tissue levels of immune complexes and various cytokines (including IFN-γ, IL-12, and TNF), activation may result from these factors. We and others have proposed potential final effectors of disease; these include arachidonic acid metabolites, ROS, and 'NO (66–68). Humans with Fas gene mutations, defective apoptosis, and autoimmune abnormalities have been recently described (69,70).

'NO, OONO−, Inflammation, and Arthritis

We noted earlier that the macrophages of MRL-lpr/lpr mice are activated in various ways, including enhanced ROS generation (67). We hypothesized that they might also overproduce 'NO (66), and we demonstrated that they spontaneously excrete 5 to 10 times more urinary nitrate/nitrite (stable oxidation products of 'NO) than normal mice. They have enhanced expression of ROS2 mRNA and protein in macrophages, liver, kidney, and spleen. ROS2 maps to mouse chromosome 11 (corresponding to human 17p21); this location is different from sites previously determined to be linked to disease susceptibility [chromosome 19 (fas), 7 and 12], making it unlikely that a defect in the ROS2 gene is the cause of this autoimmune disorder. Treatment of the mice with NMMA orally blocks the 'NO overproduction, and it prevents or blunts the development of arthritis, nephritis, and vasculitis, indicating that 'NO is causally related to the disease. Despite marked improvement in renal function and histology, drug treatment does not reduce the serum levels of anti-DNA antibody or alter deposition of immune complexes. Other workers have corroborated our findings of overproduction of 'NO in these MRL-lpr/lpr mice and extended the findings to another strain of mice with autoimmune disease (i.e., New Zealand white/New Zealand black) (71,72).

'NO binds to Hb tightly to form nitrosylhemoglobin (NOHb). This NOHb can be accurately detected by electron paramagnetic resonance techniques (EPR). Nonhuman animals in septic shock and humans receiving intravenous nitroglycerin have detectable levels of NOHb (73). We measured NOHb in whole blood. MRL-lpr/lpr mice had statistically significant elevated levels of NOHb in blood (74). This increased with age and paralleled the course of 'NO overproduction. We also examined the kidneys using EPR and found that MRL kidneys have a nitrosyl nonheme iron protein signal, as well as some NOHb (probably from blood trapped in the kidney). The nitrosyl nonheme iron protein at g = 2.04 may be an iron–sulfur cluster protein such as one of the mitochondrial electron transport enzymes.

Our studies showed that kidneys from MRL mice have an increase in the amount of nitrotyrosine-containing proteins. Nitrotyrosine is formed as a consequence of action of OONO− on tyrosine in proteins, and thus may serve as a marker of 'NO and OONO− action in tissues (75–77). On immunoblots using specific antinitrotyrosine antibody, extracts from kidneys from normal mice were essentially negative, whereas those from MRL mice had two major bands of immunoreactivity (M, 60,000 and 48,000) and three minor bands (78). Reactivity was eliminated by omitting the primary antibody or by coincubating the primary antibody reaction mixture with 10 mM nitrotyrosine. The identity of the nitrated proteins in the tissues from the diseased kidneys is presently unknown. 'NO and OONO− can react with numerous different proteins, and these reactions can alter functions of some (9,10). In an attempt to identify one of the target proteins for 'NO and OONO−, we measured catalase activity in the mouse kidneys. Catalase levels were diminished in MRL mice, and this decrease was prevented by treating them with NMMA in vivo. In in vitro studies, we noted that OONO− would inactivate purified catalase. This suggested that catalase is one of the target proteins inactivated by 'NO and/or OONO−. All this indicates that catalase
can serve as a target for OONO⁻-mediated modification, and that the modified protein has decreased activity. Thus, in the MRL mice, there is overproduction of oxidants ("NO, O₂⁻", and ONOO⁻") with depletion of the antioxidant enzyme catalase. This produces the setting of extreme oxidant stress. Evidence of increased lipid peroxidation and oxidant stress in these mice has been reported (79).

**Targeted Disruption of the Genes for NOS2**

Studies using homologous recombination between incoming DNA and a chosen target gene (gene targeting) in embryonic stem cells to make planned changes in the mouse germ line have allowed production of desired knockout (KO) mice. One can study the effects of the absence of genes and their products. KO models for the three isoforms of NOS [NOS1 (80), NOS2 (81–83), and NOS3 (84)] have been reported. Mice of each of these KOs develop normally, reproduce, and appear grossly normal. The NOS1 KO mice have gastropathy due to absence of "NO-releasing neurons in the pylorus of the stomach, and they are overly aggressive. NOS3 KO mice have hypertension (84). NOS2 KO mice do not elevate levels of "NO after immune stimulation or after LPS injection, and their macrophages cannot make NOS2 protein or "NO (81,83). They have enhanced susceptibility to disseminated infection with Leishmania and *Listeria monocyogenes*, and anesthetized mice may have increased lethality after challenge with high doses of LPS (especially those mice who have previously been injected with *Corynebacterium parvum*).

One group has shown that awake, untreated NOS2 KO mice are not resistant to LPS-induced death (82), suggesting that other factors are operative in this complex model of shock. NOS2 KO mice have diminished paw swelling after injection of carrageenin, and their lymphocytes show increased production of Th2 cytokines after stimulation in vitro (83). NOS2 KO mice have reduced resistance to infection with *Leishmania major* and *Mycobacteria tuberculosis* (83,85).

**NOS2 Knockout Mouse Studies in Autoimmune Mice**

We have crossed the NOS2 KO mice with MRL mice, and these are to four backcrosses (N4). Because the embryonic stem cells are the 129 strain, the targeted mice are not genetically homogeneous, but N4 mice have diluted out most of 129 strain of the embryonic stem cells and express essentially the MRL background. MRL-lpr/lpr littermates homozygous for disrupted NOS2 ("/-"), heterozygous for disrupted NOS2 ("+/"), or no disruption of NOS2 ("+/+)") were derived for this study (86).

The (+/-) mice excreted large amounts of nitrite/nitrate, confirming our prior observations (66). The (-/-) mice excreted intermediate levels, whereas ("/-") mice excreted very low levels of nitrite/nitrate (comparable to those of normal BALB/c mice). Nitrite/nitrate levels in sera from 20-week-old animals paralleled the urinary measures, with very low levels in the ("/-") mice, high levels in the (+/-) mice, and intermediate levels in the (+/-) mice. Levels of urinary and serum nitrite/nitrate in (+/-) or (-/-) mice were significantly higher than those in the ("/-") mice (86).

To assess in vitro "NO production by cells from mice of the three groups, we cultured peritoneal macrophages without additives and with IFN-γ (50 U/ml) and LPS (10 ng/ml). Nitrite/nitrate levels were significantly lower in the tissue culture supernatants of macrophages from the ("/-") mice than (+/-) mice or the (+/-) mice, both at baseline and following stimulation. Similarly, NOS2 enzyme activity, as measured by the conversion of L-arginine to L-citrulline, was significantly less in the cells from ("/-") mice than those from (+/-) or (+/-) mice. NOS activities were reduced by more than 90% by inclusion of 2 mM NMA in the reaction mixtures. These studies confirm lack of detectable NOS activity in ("/-") mice (86).

Immunoblots were performed on protein extracts from spleens, kidneys, liver, and peritoneal macrophages from the mice using an anti-NOS2 antibody. The macrophage cell line J774 (no treatment or treated with LPS/IFN-γ) served as control. As noted earlier, tissue from BALB/c mice had no NOS2. There was minimal or no detectable NOS2 in the splenic, kidney and macrophage protein extracts from ("/-") mice. Extracts from (+/-) and (+/-) mice contained NOS2 protein, with those of the (+/-) mice having approximately half the amount of the ("/-") mice (86).

Based on the effect of in vivo administration of NMMA on renal disease and arthritis in MRL-lpr/lpr mice (66), we predicted that ("/-") mice would develop less renal disease and arthritis than mice of the other two groups. Twenty-four-hour urinary protein excretion at 20 weeks of age was less in the ("/-") mice than in the other two groups (but not statistically significantly different), suggesting a possible difference in renal disease. Pathologic examination, however, indicated that glomerulonephritis in the ("/-") mice was similar in severity to that of the other two groups. Proliferative glomerulonephritis was present in all mice regardless of NOS2 genotype, with overall glomerular scores similar between the groups. Crescentic glomerulonephritis and interstitial disease was present in a small number of mice in each group. Synovitis was present in most of the mice, with overall synovial scores similar in the three groups. Synovial hypertrophy, synovial inflammation, and erosive disease were present to a similar degree in the MRL-lpr/lpr mice regardless of NOS2 genotype (86).

Infiltrates of lymphocytes and perivascular collection of lymphocytes occur in the kidneys of all lpr mice regardless of genetic background (64,87); true medium-to-large vessel vasculitis is found, however, only in MRL-lpr/lpr mice (87–89). Mononuclear cell infiltrates were present in the kidneys of all mice in this study. However, in contrast to glomerulonephritis and arthritis, there was a significant difference in the presence and severity of medium-to-large vessel vasculitis, depending on NOS2 genotype. Indeed, 4 of 6 (+/-) mice had prominent vasculitis of medium-to-large vessels in the kidney, whereas only 1 of 9 of the ("/-") mice and 0 of 9 of ("/-") mice had medium-to-large vessel vasculitis. The incidence of vasculitis in the (+/-) mice was similar to that in 20-week-old female MRL-lpr/lpr mice (80%). The difference in the occurrence of vasculitis between the (+/-) mice and the ("/-") mice was statistically significant. Pathologic examination of the brain, liver, lymph nodes, spleen, and lung revealed similar mild lymphocytic infiltration in all three groups (86).

MRL-lpr/lpr mice are notable for autoantibody production (64,87). We therefore determined if MRL-lpr/lpr mice of various NOS2 genotypes displayed qualitative or quantitative differences in autoantibody production. Serum levels of antibodies to single-strand or double-strand DNA did not differ in the various mice. These results are comparable to the lack of effect of NMMA treatment on anti-DNA production we noted before in MRL-lpr/lpr mice (66). However, there was a shift in the isotype of anti-DNA antibodies produced. The IgG1/IgG2 ratio of anti-DNA antibodies was higher in the
(-/-) mice. In contrast to anti-DNA production, rheumatoid factor (RF) production differed among the MRL-lpr/lpr mice of various NOS2 genotypes. The (-/-) mice produced significantly less IgG RF than did (+/+). IgM RF levels were also lower in the (-/-) mice, but levels were not significantly different from those in (+/+). IgG RF activity known to be associated with small-vessel but not medium-vessel vasculitis in MRL-lpr/lpr mice (90), was similar in the three groups of mice. Total serum IgG and IgM were similar in the three groups, as were serum levels of anti-Sm and anti-La antibodies.

The different antibody levels in NOS2-disrupted mice may highlight the fact that small amounts of NOS can modulate B-cell function by modifying Bcl-2 levels and apoptosis (97).

The mechanism(s) for the contrasting effects on renal and synovial diseases of a genetically disrupted NOS2 as opposed to pharmacological inhibition of NOS activity with NMMA is not clear (Table 1). It is unlikely that there were compensatory increases in NOS1 or NOS3 in the (-/-) mice, since we noted very low total body 'NO production in the (-/-) mice. NMMA is an isoform-nonspecific NOS inhibitor, blocking all three isoforms of the NOS enzymes (92). Inhibiting all NOS isoforms (and hence potentially all 'NO production) with NMMA may be more effective in disease prevention than genetically disrupting only NOS2. Alternative inflammatory pathways may not be active when 'NO production is acutely blocked by NMMA; however, these pathways might become active over time when NOS2 is genetically disrupted and absent the entire life of the animal.

Conclusions

Thus, MRL-lpr/lpr mice spontaneously overexpress NOS2 and overproduce 'NO in parallel with the development of autoimmunity and inflammation. Inhibiting 'NO production in vivo by oral administration of NMMA from 10 to 20 weeks of age prevents development of glomerulonephritis, arthritis, and vasculitis. This indicates that 'NO is important in the pathogenesis of glomerulonephritis, arthritis, and vasculitis in these mice. However, in MRL-lpr/lpr mice with genetically disrupted NOS2, arthritis and glomerulonephritis are unaltered, whereas vasculitis is reduced. These studies highlight the heterogeneity and complexity of the roles of NOS2 and 'NO in inflammation in MRL-lpr/lpr mice.
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