Commentary

The Multiplex Function of Nitric Oxide in (Auto)immunity

By Christian Bogdan

From the Institute of Clinical Microbiology, Immunology and Hygiene, University of Erlangen, D-91054 Erlangen, Germany

Study of the role of nitric oxide (NO) in mammalian organisms has a history of complexities. When eukaryotic cells were demonstrated to generate NO from the aminoacid l-arginine, we were first stunned and then fascinated by the idea that a molecule with such a simple structure exerts messenger functions and regulates complex life processes. Soon, however, we had to learn that there are at least three different isoforms of nitric oxide synthases (NOS), which all catalyze the same redox reaction, but differ in biochemical and structural properties, output of NO, function, distribution, and regulation (1, 2). The introduction of the acronyms ncNOS, iNOS, and ecNOS helped us to memorize that the type 1 NOS is constitutively expressed in neurons, where its activity is regulated by Ca^{2+} gradients and is critical for neurotransmission and learning; that the type 2 NOS is transcriptionally induced by cytokines, is independent of elevations of calcium, and is prototypically expressed in inflammatory macrophages, which makes them cytotoxic against microbial pathogens and tumor cells; and that the type 3 isoform is found as a constitutive enzyme in the endothelial layer of blood vessels, thereby regulating vascular tone and adhesion of circulating blood cells. Of course, it was not long before this simplified conception of a benign and host-protective NO world was challenged by the discovery of harmful effects of NO such as neurotoxicity, reperfusion injury, and severe hypotension during endotoxic shock (1, 2).

For immunologists in particular, iNOS turned out to be both friend and foe: on the one hand iNOS-derived NO conveys protection against many (but by no means all) intracellular bacteria and parasites, helps to fight several viral infections, and is implicated in the control of malignancies (3–6). On the other hand iNOS might also promote tumor angiogenesis and metastasis (7, 8), and (i)NOS-dependent tissue destruction and/or disease has been seen in several rodent autoimmunity models, such as experimental allergic encephalitis (EAE) and uveitis (EAU), inflammatory arthritis, and immune complex glomerulonephritis (9–14). Now, a number of recent studies, published in this and other journals, again extend our ideas of NO function in (auto)immunity and infectious diseases, as highlighted below.

Protective Functions of iNOS in EAE, EAU, and Interstitial Nephritis. A frequently proposed cascade for the development of organ-specific autoimmune disease invokes the induction and expansion of Th1 in response to microbial antigens and IL-12, which secrete interferon IFN-γ and thereby activate macrophages and other effector cells for the production of tissue-damaging molecules such as reactive oxygen intermediates or NO. Several parts of this concept have been repeatedly challenged, particularly in the EAE mouse model, which shares some similarities with human multiple sclerosis. In mice in which EAE was induced via immunization with myelin basic protein (MBP) combined with microbial adjuvants, it has been uniformly shown that IFN-γ is not only dispensible for the development of encephalitis, but clearly protects against disease progression or relapses in susceptible mice and contributes to the resistance of strains in which EAE cannot be elicited (15, 16). Segal et al. recently reported in this journal that the induction of EAE in IFN-$\gamma^{-/-}$ mice can be prevented by the simultaneous administration of anti–IL-12 antibodies and that IL-$12^{-/-}$ mice are completely resistant to disease development, most likely due to the expansion of a MBP nonspecific $CD4^+$ T cell population that produces IL-10, counterregulates the encephalitogenic (EAE effector) T cells, and is itself subjected to control by IL-12. Lymph node cells from anti–IL-12–treated and immunized mice were unable to transfer the disease to naive recipients, and splenocytes from naive donors treated with anti–IL-12 suppressed the development of EAE in immunized recipients (17). The above findings argue for a disease-protective role of IFN-γ and a disease-promoting function of endogenous IL-12 that becomes overt in the absence of endogenous IFN-γ. In both cases the cytokine effect might be mediated by iNOS-derived NO. Segal et al. observed high levels of TNF-α and iNOS mRNA in the spinal cords of MBP-immunized C57BL/6 IFN-$\gamma^{-/-}$ mice, which were markedly reduced after treatment with anti–IL-12 (17). This is compatible with but certainly does not prove the idea that iNOS/NO contributes to the IFN-γ–independent disease-promoting effect of IL-12. In contrast, in IFN-$\gamma^{+/+}$ PL/J mice, the pharmacologic inhibition or genetic deletion of iNOS was associated with an increased incidence and/or enhanced severity of EAE induced by immunization with MBP (18). Comparable results were also obtained by Kahl et al. (19). This strongly suggests a protective, antiinflammatory role of iNOS. Possible underlying mechanisms include known functions of iNOS/NO such as the suppression of T cell proliferation and Th1 cytokine production, the reduction of leukocyte adhesion and infiltration, the inhibition of other tissue-damaging pathways (e.g., NADPH oxidase), the scavenging of superoxide, and/or the apoptosis of macrophages or (encephalitogenic) T cells (for review see references 5 and 20; 21–24).

J. Exp. Med. © The Rockefeller University Press • 0022-1007/98/05/1361/05 $2.00
Volume 187, Number 9, May 4, 1998 1361–1365
http://www.jem.org

Table 1. *Effect of (i)NOS inhibition on the course of EAE**

Species/Strain	Inhibitor used	Effect on MBP-induced disease	Effect on adoptively transferred disease	Reference
Lewis rats	AG	exacerbation	not tested	32
	L-NMMA	no effect	no effect	
	L-NAME	no effect	no effect	
Lewis rats	L-NMMA	exacerbation	not tested	33
	L-NAME	exacerbation	not tested	
Lewis rats	AG	not tested	protection	12
Lewis rats	L-NIL	exacerbation	protection	25
SJL mice	AG	not tested	protection	11
SJL mice	iNOS antisense ODN	not tested	protection	41
SWXJ-14 mice	D609	protection	not tested	26
	c-PTIO	protection	not tested	
	uric acid	protection	not tested	
(PL/J × SJL) F_1 mice	AG	protection	protection	13
PL/J mice	AG	exacerbation	not tested	18
(129SvEv × PL/J × PL/J mice	iNOS gene deletion	exacerbation	not tested	18
(129SvEv × C57BL/6) F_2 mice	iNOS gene deletion	exacerbation	not tested	19

AG, aminoguanidine; L-NAME, L-nitroarginine-methyl-ester; L-NMMA, L-monomethyl-arginine; L-NIL, L-iminoethyl-lysine; c-PTIO, 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide; ODN, oligodeoxynucleotide.
*Modified from reference 25.

However, the picture on the role of iNOS/NO in rodent EAE is far from uniform. The results obtained by Fenyk-Melody et al. (18) are in accordance with three studies in the rat EAE model, but at first glance contradict another set of studies with different strains of mice, in which treatment with aminoguanidine (an NOS inhibitor with relative selectivity for iNOS), D609 (an inhibitor of activity of phosphatidylcholine-specific phospholipase C), 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (c-PTIO; an NO scavenger), or uric acid (a putative scavenger of peroxinitrite) ameliorated the severity of EAE. The results of these studies are summarized in Table 1, and as recently proposed by Gold et al. (25), are best reconciled by the assumption that iNOS can exhibit two different roles in EAE, either of which might prevail depending on the mode of induction: when EAE is induced by the injection of MBP-specific T cells (adoptive transfer model), the production of NO triggered by the encephalitogenic T cells appears to be primarily tissue-damaging, whereas in EAE directly induced by immunization with MBP the main function of NO appears to be counterregulatory and disease-limiting. There is certainly an impact of the species (rat versus mouse) and the mouse strain as illustrated by the disparate results obtained by Brenner et al. (13). As to the findings of Hooper et al. (26) (Table 1), it is important to bear in mind that phosphatidylcholine-phospholipase C is the key enzyme of a signaling pathway that has many intracellular targets with no specificity for the induction of iNOS. The potent protective effect of uric acid clearly deserves further clarification, especially because the NO levels in the brains of mice treated with uric acid were prominently increased rather than decreased (26). In view of the discrepancies between the studies listed in Table 1, it would be wise to avoid the use of NOS inhibitors, which are already known to suppress general stimulatory pathways (e.g., D609), exhibit functions unrelated to NOS (e.g., aminoguanidine, which inhibits copper-containing amine oxidases, catalase, and the formation of advanced glycosylation end-products, and generates hydrogen peroxide in the presence of Cu^{2+}; 27 and references therein) or show little or no selectivity for the inducible isoform of NOS (e.g., the l-arginine analogues L-NAME and L-NMMA, which impair iNOS, ncNOS, and ecNOS activity and cause hypertension and loss of weight; 28, 29, and references therein). As a final point, iNOS-positive cells in the CNS from diseased SJL mice (or in the brain from multiple sclerosis patients) have been identified as members of the macrophage/microglia as well as astrocyte lineages (26, 30), but it remains speculative to ascribe the opposing functions of NO to these different cell types.

Protective and disease-mediating roles of iNOS/NO have also been discovered in two other autoimmune disease models, supporting the existence of a general principle. In the rat model of autoimmune interstitial nephritis, treatment with l-N^6-(1-iminoethyl)-lysine (L-NIL), a potent and relatively selective inhibitor of iNOS, intensified the renal injury (29). In EAU induced by immunization with interphotoreceptor retinoid binding protein in adjuvants, genetic deletion of iNOS or low-dose (50 mg/kg) treatment with L-NAME delayed the onset and decreased

the severity of the ocular inflammation, whereas high-dose treatment with L-NAME was previously seen to exacerbate the disease (14 and references therein; 31). These findings point to a proinflammatory effect of iNOS in EAU and illustrate the difficulty of interpreting results obtained with the nonselective NOS inhibitor L-NAME. Similar to the EAE model, EAU will develop in the absence of endogenous IFN-γ, and endogenous IFN-γ at the systemic level appears to play a disease-limiting, protective role (31a). Whether this latter effect also involves iNOS remains to be investigated.

Counterprotective Functions of iNOS in Infectious Diseases. iNOS/NO does not have universal antimicrobial potency. First, several microbial species (e.g., *Salmonella*, *Mycobaterium avium/intracellulare*, *Mycobacterium tuberculosis*) exhibit intrinsic or strain-dependent resistance to NO, the molecular basis of which has begun to be unravelled. Second, in a number of infections (e.g., *M. avium* infections, influenza virus pneumonia, rabies, or borna virus encephalitis) expression of iNOS was clearly correlated with disease progression, arguing for a proinflammatory, autotoxic, and/or immunosuppressive function of NO (for review see references 2, 4, 6). So far, iNOS appeared to be either protective or counterprotective for the course and outcome of a given infectious disease. A clear exception to this rule has now been demonstrated by Khan et al. in the C57BL/6 mouse model of *Toxoplasma gondii* infection (34). After low dose infection (20 cysts), 50% of $iNOS^{+/+}$ mice survived beyond day 90, whereas all $iNOS^{-/-}$ mice had died by day 30, which is in agreement with the results from previous studies (35 and references therein). In contrast, when the parasite inoculum was increased to 50–100 cysts, all $iNOS^{+/+}$ mice died within 12 d, but most of the $iNOS^{-/-}$ mice survived for $\geq$ 21 d. Histology revealed extensive fatty degeneration of the liver and necrosis of the distal ileum in $iNOS^{+/+}$ mice, whereas both organs were intact in $iNOS^{-/-}$ or aminoguanidine-treated wild-type mice. However, the numbers of parasites in the brain and liver of $iNOS^{-/-}$ mice were 3- or 15-fold higher compared to wild-type controls. Thus, iNOS appears to account for the tissue damage seen in the gut and liver, but simultaneously confers some protection against the parasites in the liver and the brain. As intestinal necrosis in *T. gondii*-infected wild-type mice can also be prevented by anti–IFN-γ treatment (36), the prominent induction of iNOS in the small bowel is likely to be due to the hyperexpression of IFN-γ.

Cytokine Regulation by iNOS In Vivo. There is considerable evidence from in vitro experiments that iNOS-derived NO can modulate the cytokine response of macrophages, T cells, endothelial cells, and fibroblasts. This might be due to its capacity to activate and inactivate ion channels, G proteins, protein tyrosine kinases, Janus kinases, redox sensitive kinases, and transcription factors (for review see references 37, 38). Two recent studies highlight the possibility that NO assumes a similar regulatory function also in vivo.

Hierholzer et al. (39) analyzed the function of iNOS in a murine model of hemorrhagic shock. They report that the deletion of the iNOS gene in the mouse or pharmacologic inhibition of iNOS by L-NIL in the rat reduced the degree of tissue injury in liver and lung, which in control animals occurred within 4 h of resuscitation. The authors further demonstrate that in the absence of iNOS the activation of two transcription factors (NF-κB and Stat3) was significantly reduced in the lung and liver. The same was true for the expression of IL-6 and G-CSF, which are critical components of the inflammatory response following resuscitation from shock and are thought to be controlled by NF-κB and Stat3. Although the stimulus for the induction of iNOS in this model remains to be elucidated, the data support the conclusion that iNOS serves both tissue-damaging and cytokine regulatory functions in this model.

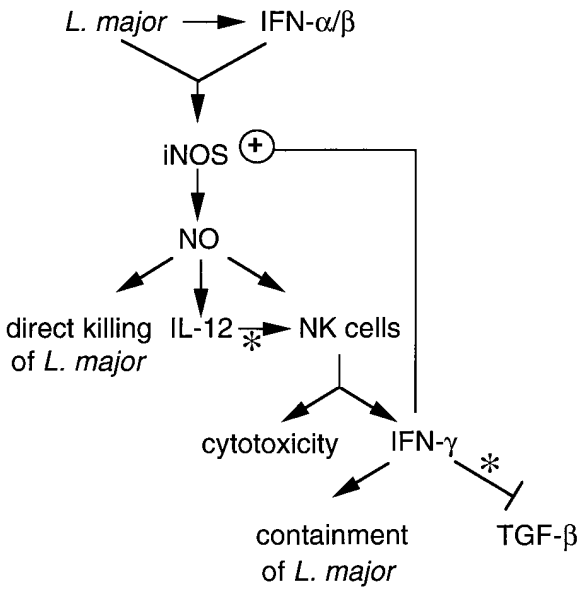

Figure 1. Scheme of putative interactions of iNOS, cytokines, and NK cells during the innate response to *L. major*. In $iNOS^{+/+}$ mice, early expression of iNOS is due to IFN-α/β, which is induced by *L. major*. NO itself can kill *Leishmania*, but also mediates (directly or via expression of IL-12 and maintaining responsiveness to IL-12) the functional maturation of NK cells (cytotoxic activity and IFN-γ production). IFN-γ, in turn, suppresses the production of TGF-β, mediates parasite containment (i.e., prevents spreading of *L. major* from the site of infection to visceral organs), and presumably further enhances the expression of iNOS. Asterisks denote processes that are dependent on endogenous NO.

In the mouse model of cutaneous leishmaniosis, iNOS was previously identified as a critical antileishmanial mechanism which was thought to start operating only when macrophages become activated by IFN-γ–secreting $CD4^+$ T cells (for review see references 4, 6). A recent study now shows that the expression of iNOS is not restricted to the T-cell–dependent late phase of infection, but is also an important component of the innate response of the host, where it is focally induced by IFN-α/β within the first 24 h of infection (40). In $iNOS^{-/-}$ or L-NIL–treated wild-type mice, there is a 30-fold reduction of the baseline expression of IL-12 p40 mRNA, an almost complete lack of the upregulation of IFN-γ, markedly reduced cytotoxic activity of NK cells, and an upregulation of the macrophage-inhibitory cytokine TGF-β in the *Leishmania major*–infected skin

and/or lymph node. Furthermore, in the absence of iNOS activity the parasites will disseminate (from the skin and lymph node to the spleen, liver, bone marrow, and lung), which is secondary to the lack of IFN-γ. In vitro, lymph node cells from day 1–infected mice fail to respond to IL-12 in the absence of iNOS (Diefenbach, A., M. Röllinghoff, and C. Bogdan, manuscript in preparation), and macrophages from iNOS$^{-/-}$ mice are refractory to the downregulation of TGF-β1 production by IFN-γ. Thus, the earlier recognized antileishmanial activity of iNOS during the late phase of infection now contrasts with a regulatory function of NO during the innate response to *L. major*, the potential sequence of which is summarized in Fig. 1.

Conclusions. The role of iNOS/NO in the immune system comprises both regulatory and effector functions. This first category includes immunosuppressive effects (e.g., inhibition of lymphocyte proliferation) and the modulation of the cytokine response. The second category includes immunopathologic effects (e.g., tissue destruction) and immunoprotective activities (e.g., killing of microbial pathogens or apoptosis of autoreactive T cells). The results discussed above illustrate that NO functions are not mutually exclusive. In fact, the prevailing data strongly suggest that signaling and effector functions of NO can operate in vivo in parallel, in a synergistic or antagonistic manner. Clearly, the mere detection of iNOS expression correlating directly or inversely with a clinical phenotype no longer allows us to draw firm conclusions as to its function. This makes it difficult to predict the effect of NO donors and iNOS inhibitors in a given disease. On the other hand, it is exactly this complexity that should encourage further studies on the pro- and antiinflammatory effects of NO, its cellular and tissue distribution, and the relationship between NO function and concentration in the microenvironment of inflammatory lesions. In this context it is also time to analyze the role of the neuronal and endothelial isoform of NOS in the immune system. No, there is no end yet to NO in immunology.

I thank Drs. A. Diefenbach, C. Nathan, M. Röllinghoff, and S. Stenger for their helpful comments and apologize to those authors whose work I could not cite directly due to the limits of space.

The preparation of the manuscript and the conduct of some of the studies reviewed were supported by the Deutsche Forschungsgemeinschaft (SFB263, A5).

Address correspondence to Christian Bogdan, Institut für klinische Mikrobiologie, Immunologie, und Hygiene, Friedrich-Alexander-Universität Erlangen-Nürnberg, Wasserturmstrasse 3, D-91054 Erlangen, Germany. Phone: 011-49-9131-852647; Fax: 011-49-9131-852573; E-mail: christian.bogdan@mikrobio.med.uni-erlangen.de

Received for publication 24 February 1998.

Note added in proof. The results reported by Fenyk-Melody et al. (18) were recently confirmed by U.C. Suhrbucher et al. using iNOS-deficient 129SvEv × C57BL/6 mice (*Eur. J. Immunol.* 1998. 28:1332–1338).

References

1. Michel, T., and O. Feron. 1997. Nitric oxide synthases: which, where, how, and why? *J. Clin. Invest.* 100:2146–2152.
2. Nathan, C. 1997. Inducible nitric oxide synthase: what difference does it make? *J. Clin. Invest.* 100:2417–2423.
3. Xie, K., Z. Dong, and I.J. Fidler. 1996. Activation of nitric oxide gene for inhibition of cancer metastasis. *J. Leukocyte Biol.* 797:797–803.
4. Bogdan, C. 1997. Of microbes, macrophages and NO. *Behring Inst. Res. Commun.* 99:58–72.
5. MacMicking, J., Q.-W. Xie, and C. Nathan. 1997. Nitric oxide and macrophage function. *Annu. Rev. Immunol.* 15:323–350.
6. Fang, F.C. 1997. Mechanisms of nitric oxide-related antimicrobial activity. *J. Clin. Invest.* 99:2818–2825.
7. Jenkins, D.C., I.G. Charles, L.L. Thomsen, D.W. Moss, L.S. Holmes, S.A. Baylis, P. Rhodes, K. Westmore, P.C. Emson, and S. Moncada. 1995. Roles of nitric oxide in tumor growth. *Proc. Natl. Acad. Sci. USA.* 92:4392–4396.
8. Duenas-Gonzalez, A., C.M. Isales, M. del Mar Abad-Hernandez, R. Gonzalez-Sarmiento, O. Sangueza, and J. Rodriguez-Commes. 1997. Expression of inducible nitric oxide synthase synthase in breast cancer correlates with metastatic disease. *Mod. Pathol.* 10:645–649.
9. McCartney-Francis, N., J.B. Allen, D.E. Mizel, J.E. Albina, Q.-W. Xie, C.F. Nathan, and S.M. Wahl. 1993. Suppression of arthritis by an inhibitor of nitric oxide synthase. *J. Exp. Med.* 178:749–754.
10. Weinberg, J.B., D.L. Granger, D.S. Pisetsky, M.F. Seldin, M.A. Misukonis, S.N. Mason, A.M. Pippen, P. Ruiz, E.R. Wood, and G.S. Gilkeson. 1994. The role of nitric oxide in the pathogenesis of spontaneous murine autoimmune disease: increased nitric oxide production and nitric oxide synthase expression in MRL-*lpr/lpr* mice, and reduction of spontaneous glomerulonephritis and arthritis by orally administered N^G-monomethyl-l-arginine. *J. Exp. Med.* 179:651–660.
11. Cross, A.H., T.P. Misko, R.F. Lin, W.F. Hickey, J.L. Trotter, and R.G. Tilton. 1994. Aminoguanidine, an inhibitor of inducible nitric oxide synthase, ameliorates experimental autoimmune encephalomyelitis. *J. Clin. Invest.* 93:2684–2690.
12. Zhao, W., R.G. Tilton, J.A. Corbett, M.L. McDaniel, T.P. Misko, J.R. Williamson, A.H. Cross, and W.F. Hickey. 1996. Experimental allergic encephalomyelitis in the rat is inhibited by aminoguanidine, an inhibitor of nitric oxide synthase. *J. Neuroimmunol.* 64:123–133.
13. Brenner, T., S. Brocke, F. Szafer, R.A. Sobel, J.F. Parkinson, D.H. Perez, and L. Steinman. 1997. Inhibition of nitric oxide synthase for treatment of experimental autoimmune encephalomyelitis. *J. Immunol.* 158:2940–2946.
14. Hoey, S., P.S. Grabowski, S.H. Ralston, J.V. Forrester, and J.

Liversidge. 1997. Nitric oxide accelerates the onset and increases the severity of experimental autoimmune uveoretinitis through an IFN-γ–dependent mechanism. *J. Immunol*. 159: 5132–5142.
15. Ferber, I.A., S. Brocke, C. Taylor-Edwards, W. Ridgway, C. Dinisco, L. Steinman, D. Dalton, and C.G. Fathman. 1996. Mice with a disrupted IFN-γ gene are susceptible to the induction of experimental autoimmune encephalomyelitis. *J. Immunol*. 156:5–7.
16. Willenborg, D.O., S. Fordham, C.C. Bernard, W.B. Cowden, and I.A. Ramshaw. 1996. IFN-γ plays a critical downregulatory role in the induction and effector phase of myelin oligodendrocyte glycoprotein-induced autoimmune encephalomyelitis. *J. Immunol*. 157:3223–3227.
17. Segal, B.M., B.K. Dwyer, and E.M. Shevach. 1998. An interleukin (IL)-10/IL-12 immunoregulatory circuit controls susceptibility to autoimmune disease. *J. Exp. Med.* 187:537–546.
18. Fenyk-Melody, J.E., A.E. Garrison, S.R. Brunnert, J.R. Weidner, F. Shen, B.A. Shelton, and J.S. Mudgett. 1998. Experimental autoimmune encephalomyelitis is exacerbated in mice lacking the NOS2 gene. *J. Immunol*. 160:2940–2946.
19. Kahl, K.G., J. Zielasek, S. Jung, R. Gold, H.P. Hartung, and K.V. Toyka. 1997. Aggravation of experimental autoimmune encephalomyelitis in mice deficient in inducible nitric oxide synthase. *Immunobiology*. 197:283 (abstr. K.20).
20. Liew, F. Y. 1995. Regulation of lymphocyte functions by nitric oxide. *Curr. Opin. Immunol*. 7:396–399.
21. Kröncke, K.-D., K. Fehsel, and V. Kolb-Bachofen. 1995. Inducible nitric oxide synthase and its product nitric oxide, a small molecule with complex biological activities. *Biol. Chem. Hoppe-Seyler*. 376:327–343.
22. Okuda, Y., S. Sakoda, H. Fujimura, and T. Yanagihara. 1997. Nitric oxide via an inducible isoform of nitric oxide synthase is a possible factor to eliminate inflammatory cells from the central nervous system of mice with experimental allergic encephalomyelitis. *J. Neuroimmunol*. 73:107–116.
23. Zettl, U.K., E. Mix, J. Zielasek, M. Stangel, H.P. Hartung, and R. Gold. 1997. Apoptosis of myelin-reactive T cells induced by reactive oxygen and nitrogen intermediates in vitro. *Cell. Imunol*. 178:1–8.
24. McInnes, I.B., B. Leung, X.-Q. Wei, C.C. Gemmell, and F.Y. Liew. 1998. Septic arthritis following *Staphylococcus aureus* infection in mice lacking inducible nitric oxide synthase. *J. Immunol*. 160:308–315.
25. Gold, D.P., K. Schroder, H.C. Powell, and C.J. Kelly. 1997. Nitric oxide and the immunomodulation of experimental allergic encephalomyelitis. *Eur. J. Immunol*. 27:2863–2869.
26. Hooper, D.C., O. Bagasra, J.C. Marini, A. Zborek, S.T. Ohnishi, R. Kean, J.M. Champion, A.B. Sarker, L. Bobroski, J.L. Farber, et al. 1997. Prevention of experimental allergic encephalomyelitis by targeting nitric oxide and peroxinitrite: implications for the treatment of multiple sclerosis. *Proc. Natl. Acad. Sci. USA*. 94:2528–2533.
27. Skamarauskas, J.T., A.G. McKay, and J.V. Hunt. 1996. Aminoguanidine and its pro-oxidant effects on an experimental model of protein glycation. *Free Radic. Biol. Med*. 21:801–812.
28. Stenger, S., H. Thüring, M. Röllinghoff, P. Manning, and C. Bogdan. 1995. L-N6-(1-iminoethyl)lysine potently inhibits inducible nitric oxide synthase and is superior to NG-monomethyl-arginine in vitro and in vivo. *Eur. J. Pharmacol*. 294:703–712.
29. Gabbai, F.B., C. Boggiano, T. Peter, S. Khang, C. Archer, D.P. Gold, and C.J. Kelly. 1997. Inhibition of inducible nitric oxide synthase intensifies injury and functional deterioration in autoimmune interstitial nephritis. *J. Immunol*. 159: 6266–6275.
30. Tran, E.H., H. Hardin-Pouzet, G. Verge, and T. Owens. 1997. Astrocytes and microglia express inducible nitric oxide synthase in mice with experimental allergic encephalomyelitis. *J. Neuroimmunol*. 74:121–129.
31. Goureau, O., B. Thillaye-Goldenberg, M.C. Naud, Y. Courtois, and Y. de Kozak. 1998. Downregulation of experimental autoimmune uveoretinitis expression in mice lacking nitric oxide synthase type II. International Symposium on Ocular Immunology and Inflammation, Amsterdam, June 1998. (abstr.)
31a. Jones, L.S., L.V. Rizzo, R.K. Agarwal, T.K. Tarrant, C.-C. Chan, B. Wiggert, and R.R. Caspi. 1997. IFN-γ-deficient mice develop experimental autoimmune uveitis in the context of a deviant effector response. *J. Immunol*. 158:5997–6005.
32. Zielasek, J., S. Jung, R. Gold, F.Y. Liew, K.V. Toyka, H.P. Hartung, and K.V. Toyka. 1995. Administration of nitric oxide synthase inhibitors in experimental autoimmune neuritis and experimental autoimmune encephalomyelitis. *J. Neuroimmunol*. 58:81–88.
33. Ruuls, S.R., S. van der Linden, K. Sontrop, I. Huitinga, and C.D. Dijkstra. 1996. Aggravation of experimental allergic encephalomyelitis (EAE) by administration of nitric oxide (NO) synthase inhibitors. *Clin. Exp. Immunol*. 103:467–474.
34. Khan, I.A., J.D. Schwartzman, T. Matsuura, and L.H. Kasper. 1997. A dichotomous role for nitric oxide during acute *Toxoplasma gondii* infection in mice. *Proc. Natl. Acad. Sci. USA*. 94:13955–13960.
35. Scharton-Kersten, T.M., G. Yap, J. Magram, and A. Sher. 1997. Inducible nitric oxide is essential for host control of persistent but not acute infection with the intracellular pathogen *Toxoplasma gondii*. *J. Exp. Med*. 185:1261–1273.
36. Liesenfeld, O., J. Kosek, J.S. Remington, and Y. Suzuki. 1996. Association of $CD4^+$ T cell–dependent, interferon γ–mediated necrosis of the small intestine with genetic susceptibility of mice to peroral infection with *Toxoplasma gondii*. *J. Exp. Med*. 184:597–607.
37. Lander, H.M. 1997. An essential role for free radicals and derived species in signal transduction. *FASEB (Fed. Am. Soc. Biol.) J*. 11:118–124.
38. Duhé, R.J., G.A. Evans, R.A. Erwin, R.A. Kirken, G.W. Cox, and W.L. Farrar. 1998. Nitric oxide and thiol redox regulation of Janus kinase activity. *Proc. Natl. Acad. Sci. USA*. 95:126–131.
39. Hierholzer, C., B. Harbrecht, J.M. Menezes, J. Kane, J. MacMicking, C.F. Nathan, A.B. Peitzman, T.R. Billiar, and D.J. Tweardy. 1998. Essential role of induced nitric oxide in the initiation of the inflammatory response after hemorrhagic shock. *J. Exp. Med*. 187:917–928.
40. Diefenbach, A., H. Schindler, N. Donhauser, E. Lorenz, T. Laskay, J. MacMicking, M. Röllinghoff, I. Gresser, and C. Bogdan. 1998. Type 1 interferon (IFN-α/β) and type 2 nitric oxide synthase regulate the innate immune response to a protozoan parasite. *Immunity*. 8:77–87.
41. Ding, M., M. Zhang, J.L. Wong, N.E. Rogers, L.J. Ignarro, and R.R. Voskuhl. 1998. Antisense knockdown of inducible nitric oxide synthase inhibits induction of experimental autoimmune encephalomyelitis in SJL/J mice. *J. Immunol*. 160: 2560–2564.