Commentary

The Multiplex Function of Nitric Oxide in (Auto)immunity

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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 amino acid 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 Ca²⁺ 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 shown that IFN-γ is not only dispensable 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-γ−/− 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-γ−/− 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/N0 contributes to the IFN-γ-independent disease-promoting effect of IL-12. In contrast, in IFN-γ−/− 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/N0 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 (see review see references 5 and 20, 21–24).
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 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-$\text{N}^6$-(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-NAM E delayed the onset and decreased

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

<table>
<thead>
<tr>
<th>Species/Strain</th>
<th>Inhibitor used</th>
<th>Effect on MBP-induced disease</th>
<th>Effect on adoptively transferred disease</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lewis rats</td>
<td>AG</td>
<td>exacerbation</td>
<td>not tested</td>
<td>32</td>
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<tr>
<td></td>
<td>L-NMMA</td>
<td>no effect</td>
<td>no effect</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L-NAME</td>
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<td>exacerbation</td>
<td>not tested</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>L-NAME</td>
<td>exacerbation</td>
<td>not tested</td>
<td></td>
</tr>
<tr>
<td>Lewis rats</td>
<td>AG</td>
<td>not tested</td>
<td>protection</td>
<td>12</td>
</tr>
<tr>
<td>Lewis rats</td>
<td>L-NIL</td>
<td>exacerbation</td>
<td>protection</td>
<td>25</td>
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<td>SJL mice</td>
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<td>not tested</td>
<td>protection</td>
<td>11</td>
</tr>
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<td>SJL mice</td>
<td>iNOS antisense ODN</td>
<td>not tested</td>
<td>protection</td>
<td>41</td>
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<td>SW XJ-14 mice</td>
<td>D609</td>
<td>protection</td>
<td>not tested</td>
<td>26</td>
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<td></td>
<td>c-PTIO</td>
<td>protection</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>uric acid</td>
<td>protection</td>
<td>not tested</td>
<td></td>
</tr>
<tr>
<td>(PL/J × SJL) F$_1$ mice</td>
<td>AG</td>
<td>protection</td>
<td>protection</td>
<td>13</td>
</tr>
<tr>
<td>PL/J mice</td>
<td>AG</td>
<td>exacerbation</td>
<td>not tested</td>
<td>18</td>
</tr>
<tr>
<td>(129SvEv × PL/J × PL/J) mice</td>
<td>iNOS gene deletion</td>
<td>exacerbation</td>
<td>not tested</td>
<td>18</td>
</tr>
<tr>
<td>(129SvEv × C57BL/6) F$_2$ mice</td>
<td>iNOS gene deletion</td>
<td>exacerbation</td>
<td>not tested</td>
<td>19</td>
</tr>
</tbody>
</table>

AG, aminoguanidine; L-NAM E, 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.

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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. In contrast to its anti-inflammatory properties, iNOS-induced NO does not have universal antimicrobial potency. First, several microbial species (e.g., Salmonella, M. avium/intracellulare, M. 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, 3, 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 ≥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.

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-γ 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 up-regulation 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 NO in the immune system. No, there is no end yet to NO in immunology.

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N ote added in proof. The results reported by Fenyk-Melody et al. (18) were recently confirmed by U.C. Suhr-bucher et al. using iNOS-deficient 129SvEvC57BL/6 mice (Eur. J. Immunol. 1998. 28:1332–1338).

References

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