expression. The second hypothesis is based on the fact that because tin protoporphyrin possesses a structure similar to that of heme, it may directly induce HO-1 expression, probably by binding to specific heme binding motifs and activating the gene transcription. Moreover, the authors’ observations further confirm that the activity of the protein rather than its expression or intracellular localization is required for its beneficial effects. We also agree with the authors regarding their conclusions about carbon monoxide. In fact, further studies using carbon monoxide–releasing molecules or carbon monoxide gas are required to confirm a possible interaction between carbon monoxide, nuclear factor erythroid 2–related factor 2, and nuclear factor xB under these experimental conditions. In this regard, further studies should also be performed to exclude a possible involvement of biliverdin, the other byproduct of HO activity, which has also been shown to impact on inducible nitric oxide synthase expression and activity.1,5,7

In conclusion, the authors’ observations are novel and intriguing and add a new, important piece in the complicated puzzle of the interaction between the HO–carbon monoxide and nitric oxide–nitric oxide synthase systems.

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In Reply:—We appreciate the interest of Li Volti et al. in our recently published article.1 In their letter, Li Volti et al. pointed out that our conclusions could have been strengthened if there had been data indicating the effect of heme on nitric oxide. Incidentally, we did perform these experiments, but we decided not to present these data in the article because of page limitations. We appreciate these comments and are glad to present these data in this response letter. Briefly, production of nitric oxide and expression of inducible nitric oxide synthase, i.e., the main enzyme for nitric oxide production during sepsis, were evaluated by chemiluminescence and immunoblotting assays, as we previously reported.2 The data revealed that heme significantly attenuated inducible nitric oxide synthase expression and nitric oxide production in lipopolysaccharide-stimulated macrophages (fig. 1). In addition, the effects of heme on inducible nitric oxide synthase expression could be attenuated by tin protoporphyrin, the potent heme oxygenase-1 inhibitor (fig. 1). Along with our previous reports,3–6 these data provide strong evidence to support the crucial role of heme oxygenase 1 on regulating inducible nitric oxide synthase expression and nitric oxide production during sepsis.

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Fig. 1. Representative gel photography illustrated the effects of heme on inducible nitric oxide synthase (iNOS) expression and nitric oxide production in lipopolysaccharide-stimulated murine macrophages. The effects of tin protoporphyrin (SnPP) on iNOS expression and nitric oxide production in lipopolysaccharide (LPS) plus heme-stimulated murine macrophages were also illustrated. The iNOS protein concentrations were normalized by β-actin. The nitric oxide concentrations were determined using chemiluminescence assay. Data are expressed as mean ± SD. H(50) and Hemin(50) were 100 μM heme, H(50) and Hemin(50) were 50 μM heme, H(500) and Hemin(500) were 500 μM heme; PBS = phosphate-buffered saline; S = tin protoporphyrin. *P < 0.05 compared with the PBS group. †P < 0.05 compared with the LPS group. ‡P < 0.05 compared with the LPS + Hemin(50) group. §P < 0.05, the LPS + Hemin(50) group versus the LPS + Hemin(50) + SnPP group or the LPS + Hemin(50) group versus the LPS + Hemin(50) + SnPP group.

(Accepted for publication April 23, 2007.)

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Anesthesiology 2007; 107:356

(Received for publication April 23, 2007.)