Nitric Oxide Synthase and Antimicrobial Armature of Human Macrophages

To the Editor—Recently Schneemann et al. [1] showed that in human mononuclear phagocytes high-output nitric oxide synthase (NOS) is not inducible by a variety of stimuli, including interferon-γ, endotoxin, and tumor necrosis factor-α, known inducers of NOS in other human and in murine cells, including macrophages. The conclusion that NOS is not a constituent of the antimicrobial armature of human mononuclear phagocytes agrees with earlier speculations by us and others, but there are still some reservations.

Because of their enzymic repertoire, human macrophages differ significantly from other human cells and particularly from murine cells: Although high activity of 6-pyruvoyl-tetrahydropterin-synthase is constitutively present in all these cells, activity is significantly lower in the human macrophage [2]. For that reason, human macrophages produce on stimulation with interferon-γ a 50-fold excess of neopterin derivatives compared with the amount of biopterin derivatives including tetrahydrobiopterin (BH4). In agreement with this in vitro finding, increased concentrations of neopterin are detectable in patients with a variety of disorders associated with stimulated cellular immunity, such as allograft rejection, autoimmune disorders, and human immunodeficiency virus infection [3], whereas biopterin concentrations change rarely and little. The ratio of neopterin to BH4 lies strongly on the side of BH4 in other human cells, such as fibroblasts and endothelial cells, or in murine macrophages, in which at least 50-fold more biopterin derivative than neopterin (or no neopterin at all) is detectable [2, 4].

Thus, there is not a complete lack of BH4 in human macrophages as described by Schneemann et al. [1] but a relative deficiency. Moreover, it has been demonstrated that deficiency of BH4 cannot explain the inability to detect nitric oxide (NO) production by human macrophages, because exogenous administration of BH4 did not normalize the output of NO [5]. Still the question remains whether the cytokine profiles used so far to stimulate NOS in human macrophages are incomplete and the stimuli necessary for NOS induction are more distinct from the murine system or from other human cells, as was anticipated.

The question arises of why the diversity exists of pteridine biosynthetic pathways between human macrophages and other human cells and especially murine macrophages. Recently, in vitro experiments showed that neopterin can enhance effects mediated by cytotoxic substances, such as hydrogen peroxide or hypochloric acid, at a physiologic pH. Signal intensity in a luminol assay and antimicrobial toxicity of hydrogen peroxide and hypochloric acid were enhanced up to 100-fold by addition of neopterin [6]. The data suggest that neopterin can enhance cytotoxicity in general, and it may have a pivotal role in the modulation of radical-mediated effector mechanisms of macrophages. It thus appears that neopterin production itself can replace inducible high-output NOS in the human macrophage, which retains its ability to compete with invading pathogens. We think this is an intriguing hypothesis to be tested.

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Reply

To the Editor—We appreciate the comments of Fuchs et al. [1] in response to our article. We agree that there are indeed fundamental differences in biopterin synthesis, arginine metabolism, and nitric oxide (NO) production between human and murine macrophages. This observation was the basis of our article and extended previous observations from our and other laboratories (referenced in our article [2]).

The speculation of Fuchs et al. [1] that occult cytokine profiles might be required in human macrophages to induce NO synthase (NOS) activity is provocative but cannot be supported by available data, which indicate the following: Cytokine profiles required to induce BH4 synthesis or NO production in human cells other than mononuclear phagocytes, such as hepatocytes and endothelial cells, correspond to the stimuli required

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for these effects in murine cell systems [3, 4] (unpublished data). The differences are fully expressed by nonstimulated human and murine phagocytes (i.e., without prior in vitro exposure to cytokines). None of the following induce NO production or induce changes in arginine consumption in human mononuclear phagocytes: bacteria, a complete in vitro system of cell-mediated immunity, interleukin (IL)-1, granulocyte-macrophage colony-stimulating factor, IL-4, IL-10, transforming growth factor-β, an in vitro differentiation to multinucleated giant cells by prolonged in vitro culture, or the conventional NOS inducers lipopolysaccharide, interferon-γ, and tumor necrosis factor-α. This was shown in our article.

Also, in regard to the amount of detectable BH4 in human mononuclear phagocytes, we prefer to be specific. We maintain that cytosolic BH4 is <0.03 pmol $\cdot$ mg^{-1} in human mononuclear phagocytes, which is our detection limit. Therefore, we cannot prove that BH4 is totally lacking in human macrophages. We wonder, however, whether the minuscule amounts of BH4 synthase activity reported in human monocyte preparations by Fuchs et al. stem from monocytes or form contaminating lymphocytes in their cell preparations that produce BH4 [5, 6]. In any event, the availability of BH4 does not limit NO production, as is shown in table 1 of our article and confirmed recently by others ([5] in our article).

We cannot comment on the interesting speculations on neopterin and its possible functions, which have no direct relation to our article.

The statement by Fuchs et al. that there is something like an NO output that can be "normalized" in human mononuclear phagocytes testifies to their preference for unidirectional speculations. What is a normal NO output? Is it biologically important that human mononuclear phagocytes do not produce NO on in vitro stimulation by cytokines, or is it biologically important that murine macrophages do so in vitro? Could it be that human macrophages operative in vivo against microbes or tumors produce NO but that, possibly, murine macrophages would not? Should we disregard all previous in vitro observations on phagocyte functions of mice and humans?

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Low Human T Cell Leukemia Virus Type II Seroprevalence in Africa

Colleagues—Initial surveys in sub-Saharan Africa found a high prevalence of antibodies to human T cell leukemia virus (HTLV) [1], later identified as predominantly HTLV-I. HTLV-II is known to be epidemic among intravenous drug users in the United States [2] and Italy [3] and has recently been identified among Amerindians in the United States, Panama, and Brazil [4]. Its presence in Africa, however, remains discrete, with only sporadic cases having been reported. Delaporte et al. [5], while analyzing samples with polymerase chain reaction (PCR), found several cases of HTLV-II in Gabon, and other reported cases have been found in pygmies from northeastern Zaire [6], patients in Somalia and Ethiopia [7], and blood donors in Guinea [8].

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Our study involved the collection of 11,788 serum samples from healthy subjects (7262) and patients (4526) in several west and central African countries. All samples were screened for antibodies to HTLV-I using an ELISA (Abbott Laboratories, Abbott Park, IL) or immunofluorescence test with HTLV-I–producing HuT102 cells. Positive samples by one or both assays were further tested by Western blot (WB) using HTLV-I–producing HuT102 cells as an antigen source [9]. Samples giving at least four virus-specific bands, including major gag antigens p19, p24, and env antigen gp46, were considered positive; those giving none of these bands were recorded as negative. Intermediate results were classed as "indeterminate." This first part of our study revealed that 153 (2.1%) of 7262 healthy subjects and 122 (2.7%) of 4520 patients had detectable antibodies to HTLV-I; 37 (0.5%) of 7262 healthy subjects were classified as indeterminate by WB (table 1).

Following these initial screening tests, all positive and indeterminate samples were then subjected to an ELISA that used two specific synthetic peptides (MA1 and MA2) representing epitopes of, respectively, HTLV-I and -II core proteins as previously described [10] and also to a second WB assay with recombinant proteins (MTA-1 and K-55) specific for HTLV-I and -II, respectively (HTLV 2-3; Diagnostic Biotechnology, Singapore) (table 1).