Species differences in macrophage NO production are important

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Bogdan concludes his recent review "Nitric oxide and the immune response" by stating: "the demonstration of iNOS expression by macrophages and other cell types in tissues from patients with a wide variety of infectious, autoimmune and degenerative diseases has disproved the claim that iNOS does not occur in the human immune system"[1]. The article provides the reader with a view with which we, investigators working with nonmurine macrophages, do not unequivocally agree.

Human macrophages are different from mouse and rat macrophages in their nitric oxide (NO) production, both *in vitro* and *in vivo*[2–6]. Mouse macrophages synthesize large amounts of NO from the substrate L-arginine, synthesize the obligate cofactor tetrahydrobiopterin (H_4B) and use arginase as the main enzyme metabolizing L-arginine—enzyme systems not functional in human macrophages[7,8]. These pathways are present in all cell types that produce NO *in vitro* and *in vivo*, including human hepatocytes and human smooth muscle cells[9,10].

The profound differences among species are not confined to human and mouse macrophages. Caprine, lapine and porcine macrophages also do not generate NO, whereas bovine macrophages do[11]. Fundamental species differences are common in immunology and other disciplines[12,13].

None of the articles describing patient material that were cited by Bogdan specify the location and identity of the cell type responsible for NO synthesis[1]. Whenever a specific cell type is proposed to have inducible nitric oxide synthase (iNOS), or any type of enzyme activity, the questions of substrate (L-arginine) consumption, cosubstrate (L-citrulline) production and cofactor (H_4B) availability or production have to be answered simultaneously. The cited articles do not address these issues. Documentation of the presence of mRNA or protein on its own is not proof of functional enzyme activity. Recently, Kun *et al*. described a point mutation in the iNOS promoter in Africans that correlates with protection against malaria[14]. It remains to be investigated whether the complete iNOS-H_4B-arginase pathway is functional in purified macrophages from subjects carrying this mutation.

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Response

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Schneemann and Schoedon disagree with my statement: "the demonstration of iNOS expression by macrophages and other cell types in tissues from patients with a wide variety of infectious, autoimmune and degenerative diseases has disproved the claim that iNOS does not occur in the human immune system"[1]. They argue that my review[1] ignored fundamental differences between murine and nonmurine macrophages and that none of the cited articles on the expression of iNOS in humans provided information on the cell types that release NO. For several reasons I find their criticism unjustified.

First, as stated in the introduction, my review does not focus on differences in the expression and regulation of iNOS in human *versus* murine cells because a number of previous reviews (which are cited) had already addressed this issue in detail and discussed all relevant publications[2–5]. Second, the important regulation of iNOS activity by arginase, arginine and cofactor availability is discussed on p. 910 of my review. However, this mode of regulation is by no means restricted to human macrophages, but also occurs in murine cells. The detection of iNOS mRNA or protein certainly does not prove enzyme activity, but this limitation also applies to the numerous studies with mouse tissues. Third, the studies with patients that I referred to on p. 913 (by citing original papers or reviews) did, indeed, identify the cells expressing iNOS. For example, iNOS protein and/or activity was found in bronchoalveolar macrophages of patients with pulmonary tuberculosis or in blood monocytes isolated from patients with hepatitis C after *in vivo* treatment with IFN-α[6,7]. Fourth, induction of iNOS in human monocytes and macrophages has undoubtedly been more difficult than in rodent cells. However, much of the frustration resulted from stimulation with IFN-γ and lipopolysaccharide[2]. As reviewed previously[4,5], alternative stimuli (such as IFN-α/β, IL-4 + anti-CD23 or chemokines) appear to be much more effective[7–10]. Fifth, irrespective of the actual amount of NO produced by human monocytes and macrophages *in vivo*, it is important to bear in mind that small quantities of NO (below the detection limit of the Griess assay and produced by iNOS or one of the constitutive isoforms of NOS) might still exert immunologically relevant functions, including antimicrobial activity[11]. Finally, my statement on the expression of iNOS in humans specifically referred to macrophages and other cell types. Human blood mononuclear cells, neutrophils, keratinocytes, epithelial cells, synoviocytes, chondrocytes, hepatocytes, neurons and glial cells all express iNOS mRNA, protein and/or activity in various disease states[5,12]. I therefore stand by the conclusions I drew.

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