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Reply

To the Editor—We appreciate the comments of Eizirik [1] in response to our article and the subsequent correspondence [2–4]. He restates the hypothesis that occult cytokine profiles are required to induce a high output of nitric oxide (NO) synthase activity (iNOS) in human mononuclear phagocytes. He observes that the basal production of 7.1 pmol of NO/islet in unstimulated human islet cells is increased to 16.5 pmol by treatment with tumor necrosis factor-α (TNFα), to 23.7 pmol by TNFα combined with interferon-γ (IFN-γ), and to 37.4 pmol by TNFα combined with IFN-γ and interleukin-1β (IL-1β) [5]. Thus, he proposes that IL-1β, by the way a secretory product of macrophages (but not of islet cells) activated with lipopolysaccharide, IFN-γ, TNFα, bacteria, or a complete afferent loop of cell-mediated immunity, could be essential for inducing iNOS activity in human mononuclear phagocytes. Our experiments [2] (table 1) tell otherwise.

Recently it was proposed that human mononuclear phagocytes can be induced to secrete NO by coculture with certain tumor cells [6]. In these studies, however, no attempts were made to control for an activation of iNOS in the tumor cells used (that had a basal NOS activity if cultured without phagocytes) by secretory products of macrophages such as IL-1β or TNFα

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Table 1. Comparison of nitrite (NO_2^-) secretion by macrophages from different human sources.

Cell source (human)	Pretreatment for 48 h	NO_2^- (μmol/10^6 cells/day)
Blood-derived macrophages	IFN-γ + GM-CSF + TNFα*	<0.1
	Autologous sensitized lymphocytes + PPD + IL-2	<0.1
	10^8 heat-killed *Listeria monocytogenes*	<0.1
	10^8 heat-killed *Moraxella catarrhalis*	<0.1
	IFN-γ + LPS + IL-1 + TNFα + HuH-7	<0.1
Hepatoma cells (HuH-7)	IFN-γ + LPS + IL-1 + TNFα	<0.1
Lung carcinoma cells (A 549/8)	IFN-γ + TNFα	4.9 ± 1.5
Peritoneal macrophages	IFN-γ + IL-1 + TNFα	<0.1

NOTE. Culture conditions are in [2]. Data are mean ± SE from triplicate experiments. IL-1, human recombinant interleukin 1-β, 100 units/mL; IFN, interferon; GM-CSF, granulocyte-macrophage colony-stimulating factor; TNF, tumor necrosis factor; PPD, purified protein derivative; LPS, lipopolysaccharide.

* Pretreatment for 12 days.

during coculture. This control is essential in view of the responsiveness of NOS in human tumor cell lines to such stimuli [7] (table 1).

We therefore conclude that in human mononuclear phagocytes, iNOS is not induced by IL-1β. In addition, in clear distinction to other human cell types, no convincing evidence has been provided for iNOS in human macrophages thus far.

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High Rate of Reactivation of Human Herpesvirus 6 in Children with Dengue Hemorrhagic Fever

Colleagues—In 1986, a new herpesvirus, now designated as human herpesvirus 6 (HHV-6), was isolated from patients with lymphoproliferative disorders [1] and was recognized to be the causative agent of exanthem subitum in 1988 [2]. It is now believed that HHV-6 persists in the host after primary infection and can be reactivated later in life. By characterization of isolates obtained worldwide from various diseases, HHV-6 is now classified into two variants: HHV-6A and HHV-6B [3].

Dengue fever is a common childhood disease in tropical countries. Although dengue virus infection usually results in mild disease, sometimes infection by this virus can result in a severe disease such as dengue hemorrhagic fever (DHF) and dengue shock syndrome [4]. Alterations in the immune system of DHF patients are likely since there is extensive damage to the lymphoid tissue of various organs and some depression of the bone marrow elements [5]. In the present study, we investigated HHV-6 reactivation in DHF patients.

The study included 30 patients with suspected DHF who were admitted to the children's ward of Charoenkrung Pracharak Hospital. Patients were 3–13 years old (3–5, 26%; 6–10, 48%; and 11–13, 26%). These patients were classified into DHF grade I or II according to their typical clinical manifestations of the following: fever, hemorrhagic manifestations including a positive tourniquet test and some bleeding phenomena, thrombocytopenia, and hemoconcentration. During the course of illness, they did not receive blood transfusion or steroid therapy. For virologic and serologic studies, peripheral blood (~5 mL) was drawn into heparinized medium during the acute phase on the first day of hospital admission and again during the convalescent phase 2–3 weeks after the onset of disease. Heparinized blood samples from both phases were separated into plasma and peripheral blood mononuclear cells (PBMC) using Ficoll-Paque (Pharmacia, Piscataway, NJ) gradient centrifugation. PBMC were divided into two parts (~2×10^6 cells each) for virus isolation and detection of HHV-6 DNA. Antibody to HHV-6 was determined by immunofluorescence antibody (IFA) testing as described [2]. The titer of antibody was expressed as the highest dilution yielding detectable immunofluorescence. Antibody to dengue virus was determined by the hemagglutination inhibition (HAI) test with dengue virus type 2 used as antigen. At least a fourfold increase in antibody titer in plasma between the acute and convalescent phases or an antibody titer of > 1:1280 in the acute phase was considered positive for a second dengue virus infection. The method for detection of HHV-6 DNA in PBMC by polymerase chain reaction (PCR) was described previously [6]. For virus isolation, PBMC were cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 0.1 unit of recombinant human interleukin-2 (provided by Takeda Chemical Industries, Osaka, Japan) and phytohemagglutinin (5 mg/mL; Honen, Tokyo) at 37°C as described [2]. The medium was changed weekly, and cells were observed for cytopathic effect (CPE) for 2 weeks. Isolates were classified by IFA test as HHV-6A or -6B by use of monoclonal antibodies OHV-1 and OHV-3; OHV-1 reacted with both variants, whereas OHV-3 reacted only with HHV-6B [7]. The Wilcoxon signed rank test was used to compare antibody titers to HHV-6 in DHF patients.

All patients had DHF with typical symptoms: fever, hemorrhagic manifestations, thrombocytopenia, and hemoconcentration. Spontaneous bleeding in some patients developed only in the tourniquet test (grade I) but others had hemorrhages, such as petechiae, maculopapular rash, gum bleeding, epistaxis, and black vomiting (grade II). Symptoms such as restlessness, hepatomegaly, abnormal reflex, vomiting, injected pharynx, and cough also appeared in some cases. All 30 suspected patients

Informed consent was obtained from parents of all patients.

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