In Vitro Testing for Antiinflammatory Properties of Compounds

To the Editor:
Singh et al. (1) recently proposed the application of the human monocyte cell line THP-1 to test for effects of potential antiinflammatory drugs and compounds. THP-1 cells were stimulated with lipopolysaccharide for 4 to 24 h, and the secretion of proinflammatory cytokines interleukin (IL)-1, IL-6, and tumor necrosis factor-α (TNF-α) was assessed. Results showed that various dietary supplements as well as pharmacologic agents significantly inhibited lipopolysaccharide-stimulated TNF-α release (1). Interestingly, this assay matches almost perfectly one we proposed that also uses THP-1 cells but uses neopterin production as a read-out for monitoring potential antiinflammatory effects of compounds (2). After publication of that report, we observed that drugs usually exert more important effects on the T-cell/macrophage interplay than on the stimulated monocytic cells themselves and thereby on the Th1-type cytokine interferon-γ, which is crucially important as a proinflammatory mediator. Unfortunately, because we used only THP-1 cells, potential effects of compounds on the T-cell population were overlooked. Accordingly, we investigated human peripheral blood mononuclear cells (PBMCs) freshly isolated from whole blood of healthy donors and stimulated them with mitogens (3–6). PBMCs were seeded at a density of 1.5 × 10^6 cells/mL and preincubated with compounds for 30 min before stimulation with phytohemagglutinin or concanavalin A. We found that a mitogen concentration of 10 μg/mL was optimal for detecting suppressive effects of compounds. Cells were incubated for 48 h at 37 °C and 5% CO₂ and supernatants were collected thereafter. Measurements of neopterin formation by methods such as ELISA and/or tryptophan degradation by HPLC were used as convenient readouts; both biochemical effects are in-

Fig. 1. Effects of sodium azide on expression, secretion, and activity of MMP-2 from hVSMCs. Densitometric analysis of gelatin zymographs (A and B) and Western blots (C and D) of cell supernatants and lysates indicated that CRP (10 mg/L), but not sodium azide (76.9 μmol/L), influences MMP-2 expression, secretion, and activity (*, P < 0.05 with CRP and not significant with sodium azide in both supernatants and cell lysates, Student–Newman–Keuls test). Each panel is representative of at least 5 experiments.

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duced by interferon-γ in human macrophages (7).

The model system of activated PBMCs has been well established in clinical immunology for several decades and allows standardization of T-cell activation and T-cell/macrophage interaction. It is certainly more informative than the myelomonocytic tumor cell line THP-1 and more relevant for in vivo testing. Our approach has already been used for testing antiinflammatory drugs for ~5 years with very reproducible results, even between assays of blood from different donors (3–6). Dose-dependent effects were detected for compounds such as resveratrol (5), drugs such as atorvastatin (4), and beverages with well-described antiatherogenic potential, such as green and black tea (3) and beer (6).

A model system that would measure products of stimulated macrophages as a read-out is clearly relevant to atherosclerosis, in which inflammation is pivotal. However, the PBMC model draws more attention to the role of T-cell/macrophage interplay, which is highly relevant in the pathogenesis of atherosclerosis, and acknowledges the existing data on the role of the proinflammatory cytokine interferon-γ in the process of atherogenesis and other inflammatory conditions. By inducing the depletion of antioxidant systems and causing oxidative stress, interferon-γ is probably the most important trigger for the production of reactive oxygen species in macrophages (8), considered to be of utmost relevance in atherogenesis. Our in vitro PBMC system is fully in accordance with the notion that macrophage products should be measured, and it is valid both in vitro and in vivo. Both neopterin production and tryptophan degradation are induced by interferon-γ in macrophages; thus, both reflect macrophage activity. In coronary heart disease, increases in neopterin concentrations not only correlate with tryptophan degradation (9) and with the activity of the disease (9), they also predict coronary events more sensitively than do methods such as the more widely used C-reactive protein measurements (10, 11). By contrast, studies on the involvement of cytokines such as IL-1, IL-6, and TNF-α in atherogenesis stem almost exclusively from in vitro experiments.

In summary, the combined study of effects on T cells and macrophages from healthy donors appears superior to using only the myelomonocytic THP-1 cell line. The alternative approach with PBMC preparations provides insight into signaling cascades, especially those initiated by T cells. The monitoring of biochemical effects such as neopterin formation and tryptophan degradation reveals more stable results in quantitative terms than does monitoring of cytokine production. Moreover, this strategy monitors the net effect of various pro- and antiinflammatory cascades initiated during stimulated immune response in vitro and in vivo and provides data on the influence of tested compounds on the whole cascade of events. Finally, both read-out systems seem particularly suited to testing for antiinflammatory effects of compounds because enhanced production of neopterin and accelerated degradation of tryptophan are closely related to the pathogenesis of various diseases in which inflammatory processes are involved.

References


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Serum Amyloid A Is Not Useful in the Diagnosis of Severe Acute Respiratory Syndrome

To the Editor:

In our present study, we aimed to investigate whether the serum concentration of serum amyloid A (SAA), as measured by the surface-enhanced laser desorption/ionization (SELDI) ProteinChip technology or by ELISA, is useful in differential-