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Immun'Âge Modulates Interferon-γ-induced Nitric Oxide Production in the Mouse Macrophage Cell Line RAW 264.7

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Summary Immun'Âge, a natural health food supplement prepared from *Carica papaya* and some other medicinal plants was investigated to determine its effects on cellular nitric oxide (nitrogen monoxide, NO) production and inducible nitric oxide synthase (iNOS) expression. Immun'Âge upregulated interferon (IFN)-γ-induced NO production by macrophages in a dose-dependent manner. Such an effect of Immun'Âge on NO production was not due to changes in the activity of iNOS. Reverse transcription-polymerase chain reaction analysis revealed that the levels of iNOS mRNAwere augmented by treatment of the cells with Immun'Âge and IFN-γ. The ability of Immun'Âge to augment IFN-γ-induced iNOS mRNA expression was independent of any changes on the mRNA stability. Treatment of cells with Immun'Âge alone did not affect NO production by macrophages. Tumor necrosis factor-α and interleukin-lβ are involved in the induction of iNOS gene as well as the immune system. Immun'Âge augmented the mRNA expression of these cytokines in the presence of IFN-γ. This suggests that Immun'Âge is not directly involved in the expression of iNOS, but shows synergistic interaction with IFN-γ to induce NO synthesis.

Key words : nitric oxide, inducible nitric oxide synthase, Immun'Âge, RAW 264.7

Introduction

Macrophages play a significant role in the host defense mechanism by the secretion of various cytotoxic agents, including lysosomal enzymes, reactive oxygen species and reactive nitrogen intermediates (1) (2). Among the reactive nitrogen intermediates, nitric oxide (nitrogen monoxide, NO) is one of smallest active biological metabolites, with a short half-life in the order of seconds (3), Recent studies have shown that the NO is associated with the expression of microbicidal and tumoricidal activities exerted by activated macrophages (2). Furthermore, it has been shown that NO also exhibits in some instances cytotoxic activity against viruses (4) (5), and fungi (6). The production of NO from macrophages is regulated by an inducible enzyme, nitric oxide synthase (iNOS; EC 1.14.13.39) which is capable of producing NO in high amounts and for prolonged periods, It is well established that iNOS is independent of Ca^{2+} transients and exogenous calmodulin

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(7), and can be induced by cytokines such as IFN-γ and migration inhibitory factor (8). In addition, IFN-γ in combination with several agents, including tumor necrosis factor-α (TNF-α) (9), interleukin-1β (IL-1β) (10), interleukin-2 (11) and lipopolysaccharide (LPS) (12) can synergistically promote the NO production by macrophages and some other cells. As such, the induction of iNOS is primarily regulated at the level of gene transcription, although it has been shown that iNOS is up-regulated by alternative pathways, including stabilization of iNOS mRNA (post-transcriptional regulation), enhancement of translation and inhibition of the degradation of NOS protein (7).

Immun'Âge (FPP) is a natural health food supplement which is made by yeast fermentation of *Carica papaya* Linn., It is produced under strict quality control and no artificial substances are added.

Current clinical trials have reported that FPP exhibit beneficial therapeutic effect in a variety of human pathologies, including cancer, epilepsy, hepatitis, ulcer, cardiac and renal insufficiency (13), Oral administration of FPP to rats has been reported to protect the heart against injury caused by ischemia-reperfusion (14) and decrease the release of monoamine metabolites in iron-induced epileptogenic focus (15). In addition, FPP has been shown to have hydroxyl radical scavenging activity using ESR spectroscopy (16). The accumulation of thiobarbituric acid reactive substances and protein carbonyl derivatives were found to be lower in heart homogenates from FPP supplemented rats exposed to peroxyl radical generator as compared to non-supplemented ones (14) (17). From such evidence, it has been proposed that the protective effects of FPP in various pathologies might be due to its free radical scavenging properties, i) FPP has also been reported to upregulate phorbol ester-induced and zymosan-induced superoxide production in rat peritoneal macrophages (18), ii) natural killer cell activity (19), and iii) the level of IFN-γ in human blood (20). Such evidence suggests that FPP also possesses immuno-modulating activity besides its direct antioxidant activity. However, despite accumulating data on beneficial effects of FPP application, little is known about the molecular mechanisms involved in its action. Reactive nitrogen intermediates such as NO, have been suggested to participate in the immune system, as well as other diverse biological activities (2). The present study was designed to investigate whether FPP is able to alter the production of NO by macrophages using the mouse macrophage cell line RAW 264.7.