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Inhibitory effect of nicotinamide on in vitro and in vivo production of tumor necrosis factor-α

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Abstract

Nicotinamide, a pellagra-preventive factor, has multiple functions such as inhibition of poly-ADP-ribose synthetase, inhibition of inducible nitric oxide synthase, free radical scavenging and suppression of major histocompatibility complex class II expression and ICAM-1 expression on endothelial cells. In addition to these, we have found an inhibitory effect of nicotinamide on production of tumor necrosis factor-α (TNF-α) in vitro and in vivo. Lipopolysaccharide (LPS)-induced in vitro TNF-α production by human peripheral blood mononuclear cells, measured by enzyme-linked immunosorbent assay (ELISA), was significantly inhibited with more than 1×10^{-3} mol/l of nicotinamide, while interleukin-1-β was not inhibited and interleukin-6 was slightly inhibited even with 10^{-2} mol/l. Oral administration of nicotinamide with more than 62.5 mg/kg also significantly inhibited LPS-induced serum TNF-α production measured by ELISA and bioassay in Balb/c mice. Thus, nicotinamide has an inhibitory effect on TNF-α production that may be beneficial to TNF-α-mediated diseases. © 1997 Elsevier Science B.V.

Keywords: Nicotinamide; Tumor necrosis factor-α; Pancreatic β-cell; Insulitis; Insulin-dependent diabetes mellitus

1. Introduction

Nicotinamide, a factor of vitamin B2 complex, is known as a pellagra-preventive factor. Recently it has been reported that nicotinamide has protective effects on the pancreatic β-cell in insulin-dependent diabetes mellitus (IDDM) [1] by various possible mechanisms; inhibition of poly-ADP-ribose synthetase [2], inhibition of inducible nitric oxide synthase (iNOS) [3], free radical scavenging [4] and suppression of major histocompatibility complex class II expression [5] and ICAM-1 expression on endothelial cells [6]. Based on the preventive effect of nicotinamide on diabetes in the NOD mouse [7], an animal model of IDDM, clinical trials of nicotinamide on prevention of human IDDM are in progress worldwide [8,9].

During the study on drugs for inhibition of tumor necrosis factor-α (TNF-α) production [10], we have found that nicotinamide inhibited production of TNF-α in vitro and in vivo. Here we report TNF-α inhibition with nicotinamide which may be relevant to its beneficial effect on the β-cell.

2. Materials and methods

Nicotinamide (Sigma, St. Louis, MO) was dissolved in phosphate-buffered saline (PBS) at a concentration of 1×10^{-1} mol/l and then diluted with PBS to appropriate concentrations.

Peripheral blood mononuclear cells (PBMC) were isolated from heparinized blood obtained from five healthy donors by density centrifugation and suspended in RPMI-1640 medium (Life Technologies, Grand Island, NY) supplemented with 10% heat-inactivated fetal calf

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serum (Bio WHITTAKER, Walkersville, MD), sodium pyruvate (Life Technologies) and antibiotics (Penicillin-Streptomycin-Fungizone, Bio WHITTAKER). PBMC (2×10^6 cells/ml) in 0.2 ml aliquot were cultured in 96 well flat-bottomed tissue culture plates. At the start of culture, lipopolysaccharide (LPS, *E. coli* 055:B5 Difco, Detroit, MI) at a final concentration of 20 μg/ml, which was the optimal concentration obtained by a preliminary experiment and nicotinamide with various concentrations were added to wells. The supernatants were harvested from each well at serial time points and stored at −80°C for assay of human TNF-α, interleukin-1-β (IL-1-β) and interleukin-6 (IL-6).

Male, 8–10-week-old Balb/c mice (purchased from Clea Japan, Tokyo, Japan) were intraperitoneally injected with LPS (2 mg/kg) in sterile PBS and blood was taken by cutting the axillary artery and vein under ether anesthesia at serial time points. Serum was isolated and stored at −80°C for TNF-α assay. At serial time points before LPS injection, 0.2 ml of nicotinamide/PBS or PBS was orally administered by a syringe.

TNF-α, IL-1-β and IL-6 immunoreactivity was measured by enzyme-linked immunosorbent assay (ELISA) using the commercial kit (R&D Systems, Minneapolis, MN). TNF-α activity was bioassayed by using LM cells, a subline of TNF-α-sensitive mouse fibroblast (L929) as a target cell and by using recombinant human TNF-α as a standard as previously reported [11]. Serum was finally diluted to more than 1:12 in the LM cell culture for the TNF-α assay.

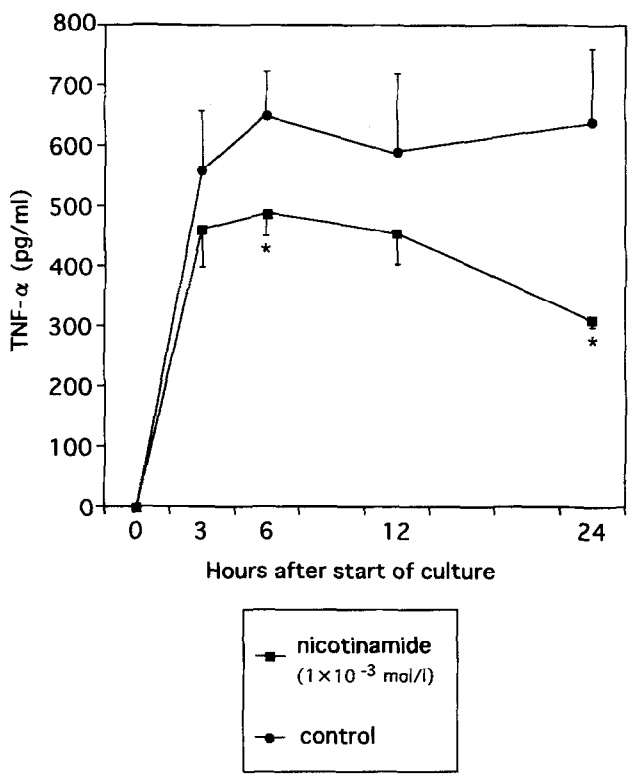

Fig. 1. Effect of nicotinamide on LPS-induced in vitro TNF-α production by human PBMC at different time points after LPS challenge. Human PBMCs were cultured with nicotinamide (1×10^{-3} mol/l) and LPS (20 μg/ml). Supernatants were harvested at serial time points and measured for TNF-α by ELISA. Values are means ± S.E. ($n = 4$). * $P < 0.05$ vs. control.

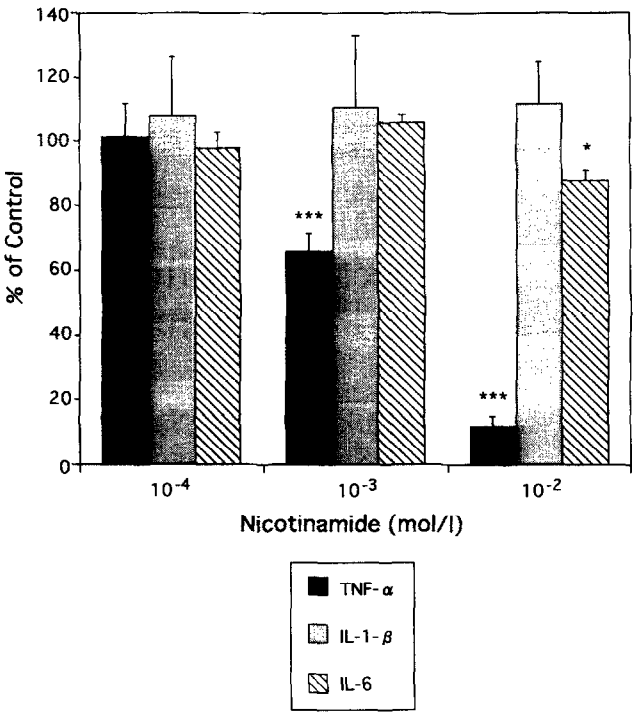

Fig. 2. Effect of nicotinamide on LPS-induced in vitro production of TNF-α, IL-1-β and IL-6 by human PBMC. PBMC were cultured in the same condition as that in Fig. 1 and supernatants were harvested after 6 h culture and measured for TNF-α, IL-1-β and IL-6 by ELISA. Results are expressed as percentage of cytokine concentrations in comparison with those of the control culture without nicotinamide and represent mean ± S.E. from the five independent experiments. * $P < 0.05$; *** $P < 0.005$ vs. control.

Statistical significance was calculated by the paired Student's t-test for the in vitro experiment and by non-paired Student's t-test for the in vivo experiment.

3. Results

3.1. Effect of nicotinamide on in vitro production of TNF-α, IL-1-β, and IL-6

Human PBMC were cultured with LPS and nicotinamide (1×10^{-3} mol/l), and supernatant was measured for TNF-α by ELISA at serial time point after the start of culture. As shown in Fig. 1, nicotinamide significantly inhibited TNF-α production after 6 h culture although the inhibition was not significant at 12 h ($P < 0.1$). Next, inhibitory effect of various concentrations of nicotinamide on LPS-induced TNF-α, IL-1-β and IL-6 production was examined at 6 h-culture. As shown in Fig. 2, nicotinamide significantly inhibited

LPS-induced TNF-α production in a dose-dependent manner at concentration of more than 1×10^{-3} mol/l, whereas it slightly inhibited IL-6 production at a high dose (1×10^{-2} mol/l) but did not affect IL-1-β production. Cell viabilities were more than 95% in these cultures.

3.2. Effect of nicotinamide on in vivo TNF-α production

We further examined the effect of nicotinamide on in vivo production of TNF-α. First, time course of LPS-induced in vivo TNF-α production was observed. Blood was obtained at serial time points after LPS challenge from the mice which were orally administered nicotinamide (250 mg/kg) 1 h before LPS challenge. As shown in Fig. 3, LPS-induced serum TNF-α production reached peak at 90 min and nicotinamide administration markedly inhibited serum TNF-α at all of the time points. Then effect of timing of nicotinamide administration on LPS-induced TNF-α production was examined. Mice were orally administered nicotinamide (250 mg/kg) 5, 3 and 1 h before LPS challenge and 90 min after LPS challenge mice were bled. As shown in Fig. 4, nicotinamide administration 1 h before LPS challenge most effectively inhibited LPS-induced TNF-α production. Finally, the effect of the dose of nicotinamide on LPS-induced in vivo TNF-α production was observed in the mice which were administered nicotinamide 1 h before LPS challenge and bled 90 min after LPS challenge. As shown in Fig. 5A, nicotinamide significantly inhibited LPS-induced TNF-α production measured by ELISA in a dose-dependent manner at a dose of more than 62.5 mg/kg. The similar inhibitory effects were obtained by bioassay of TNF-α (Fig. 5B).

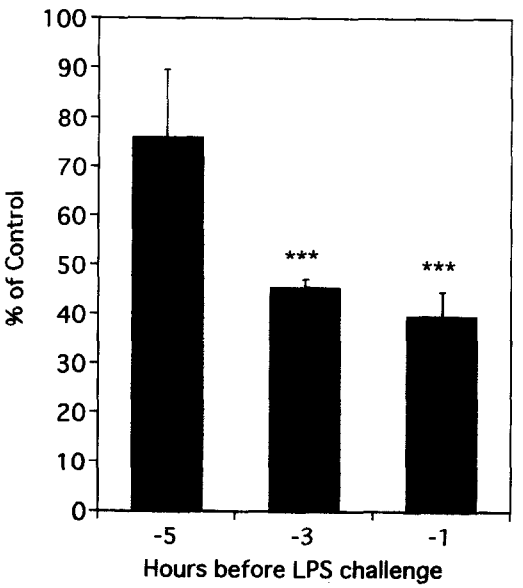

Fig. 4. Time course of inhibitory effect of nicotinamide on LPS-induced in vivo TNF-α production. Balb/c mice were orally administered nicotinamide and then 5, 3, 1 h before intraperitoneal LPS challenge (2 mg/kg). Ninety min after LPS challenge, the mice were bled and serum was measured for TNF-α by ELISA. Results were expressed as percentage TNF-α of control mice. Values are means ± S.E. ($n = 7 - 8$). *** $P < 0.005$ vs. control.

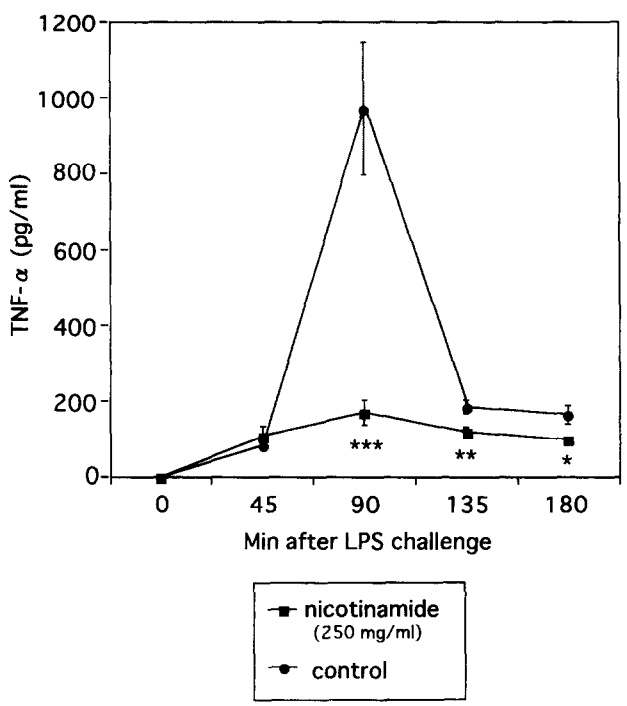

Fig. 3. Effect of nicotinamide on LPS-induced in vivo TNF-α production at different time-points after LPS administration. Balb/c mice were orally administered nicotinamide (250 mg/kg) or sterile water and 1 h later the mice were intraperitoneally injected with LPS (2 mg/kg). After 45, 90, 135 and 180 min, the mice were bled at serial time points and serum was measured for TNF-α by ELISA. Values are means ± S.E. ($n = 6 - 8$). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$ vs. control.

4. Discussion

LPS-induced TNF-α in the PBMC culture was significantly inhibited by the presence of nicotinamide (Fig. 2), indicating that nicotinamide inhibited production of TNF-α and/or release of TNF-α from cells. Recently it has been reported that inhibition of ADP-ribosylation suppresses LPS-induced change of phosphorylation of two cytosolic proteins and production of TNF-α and IL-6, but not IL-1 in human monocytes [12]. This report consists of our observations and indicates that TNF-α production rather than TNF-α release might be inhibited in our in vitro experiment. The reason why production of IL-1-β and IL-6 were slightly inhibited with nicotinamide is unclear so far, although these cytokines as well as TNF-α are produced by monocytes.

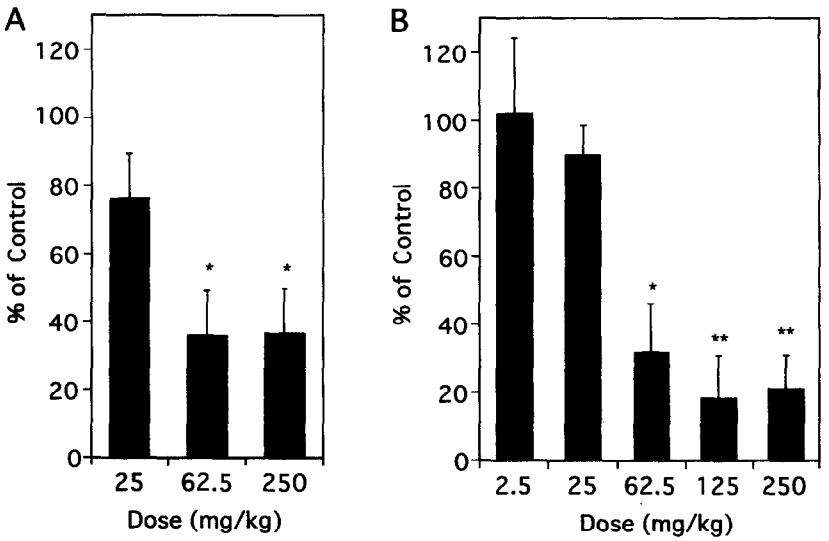

Fig. 5. Effect of various nicotinamide doses on LPS-induced in vivo production of TNF-α. The mice were administered various doses of nicotinamide 1 h before LPS challenge and bled 90 min after LPS challenge. Serum was measured for TNF-α by ELISA (A) and bioassay (B). Results are expressed as percentage of immunoreactivity (A) or bioactivity (B) of the serum TNF-α of control mice and represent mean ± S.E. from the five independent experiments. * $P < 0.05$; ** $P < 0.01$ vs. control.

Next we found inhibitory effect of nicotinamide on in vivo production of TNF-α (Fig. 5A and B), which was measured by both ELISA and bioassay. Nicotinamide per se inhibited in vitro LM cell-cytotoxicity of TNF-α at a high concentration of more than 1×10^{-2} mol/l (data not shown). Therefore, it cannot be denied that serum nicotinamide might suppress bioassay of TNF-α. However, serum TNF-α measured by not only bioassay but also ELISA was inhibited in the nicotinamide-administered mice (Fig. 5A and B), indicating that nicotinamide inhibited production of TNF-α in vivo. The doses of nicotinamide which inhibited the in vivo TNF-α production of the mice were compatible to those which are clinically used and indicated to induce remission of newly-diagnosed IDDM and to prevent onset of IDDM in high risk subjects [9–11].

Although precise action mechanisms of nicotinamide in TNF-α inhibition are obscure, in addition to inhibition of ADP-ribosylation [12] some other mechanisms have been implicated in the literature. It has been reported that nicotinamide increases intracellular cAMP levels in the liver [13] and renal tubules [14] and that drugs which increase intracellular cAMP levels in macrophages inhibit TNF-α, but not IL-1-β, production [15]. Therefore, it is probable that elevation of cAMP levels might be involved in the mechanism of TNF-α inhibition with nicotinamide.

It is generally accepted that the β cell is destroyed by the antigen-specific pathway mediated by the T-cell receptor and also by the antigen-nonspecific pathway mediated by cytokines in the insulitis lesion of IDDM [16]. As to the latter pathway, IL-1-β alone or in combination with interferon-γ (IFN-γ) and TNF-α induces NO in the β cell. NO in turn damages nuclear and mitochondrial DNA, resulting in the activation of poly-ADP-ribose synthetase, deficiency of NAD and ATP, and then in cell dysfunction and death [17]. Nicotinamide inhibits these processes by the inhibition of poly-ADP-ribose synthetase [2], the inhibition of iNOS [3] and scavenging of free radicals [4].

In addition to IL-1-β, TNF-α is suggested to play a role in exacerbating insulitis and β-cell destruction in the insulitis lesion [17]. TNF-α enhances IL-1-β-induced β-cell destruction in vitro, whereas IL-1-β induces TNF-α in the β-cell per se in vitro [18]. Furthermore, it has recently been shown that TNF-α recruits inflammatory cells to the islet and promotes β-cell damage in the transgenic mouse in which costimulator B7-1 and TNF-α genes were expressed in the β-cell [19]. Therefore, we think that, in addition to the known nicotinamide actions, TNF-α inhibition of nicotinamide may also protect the β-cell in the insulitis lesion. This mechanism may be related to the inhibitory effect of nicotinamide on development of insulitis in NOD mice [7], the mechanism of which is not fully explained by the known actions mechanism of nicotinamide described above.

In summary, nicotinamide inhibits production of TNF-α in vitro and in vivo, and it may be beneficial to various TNF-α-mediated pathologic conditions.

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