*Immunology Letters*, 31 (1991) 35 – 40
Elsevier

IMLET 01712

# Inhibitory effects of nicotinamide on recombinant human interferon-gamma-induced intercellular adhesion molecule-1 (ICAM-1) and HLA-DR antigen expression on cultured human endothelial cells

Yuji Hiromatsu, Masayuki Sato, Kentaro Yamada and Kyohei Nonaka

*Division of Endocrinology and Metabolism, Department of Medicine, Kurume University School of Medicine, Fukuoka, Japan*

(Received 30 July 1991; accepted 12 August 1991)

## 1. Summary

Intercellular adhesion molecule-1 (ICAM-1), HLA-A, B, C and HLA-DR antigen on endothelial cells (EC) play important roles in the development of inflammatory processes in autoimmune disorders. In the present study, we investigated the effect of nicotinamide, an inhibitor of poly(ADP ribose) synthetase, on interferon-γ (IFNγ)-induced ICAM-1 and HLA-DR antigen expression on the surface of cultured human umbilical vein endothelial cells, assessed by flow cytometry, and EC proliferation by counting cell numbers and [$^3$H]thymidine incorporation assays. Nicotinamide dose-dependently inhibited the IFN-γ-induced ICAM-1 and HLA-DR antigen expression, but not HLA-A, B, C antigen expression on cultured EC. Furthermore, nicotinamide significantly inhibited endothelial cell proliferation, as assessed by [$^3$H]thymidine incorporation assay. Our findings suggest that nicotinamide may suppress mononuclear cell infiltration, antigen presentation and angiogenesis in the lesions of autoimmune disorders by reducing both IFNγ-induced ICAM-1 and HLA-DR antigen expression on EC, and EC proliferation. Therefore, nicotinamide can be used for the treatment and prevention of the development of autoimmune disorders.

*Key words:* Interferon-γ; Intercellular adhesion molecule; HLA-DR antigen; Poly(ADP ribose) synthetase inhibitor; Endothelial cell

*Correspondence to:* Dr. Y. Hiromatsu, Division of Endocrinology and Metabolism, Department of Medicine, Kurume University School of Medicine, 67 Asahimachi, Kurume, Fukuoka 830, Japan. Tel.: 0942 35 3311.

## 2. Introduction

Many lines of evidence show that endothelial cells play an important role in the development of inflammation, especially lymphocytic infiltrations in autoimmune disorders such as rheumatoid arthritis [1] and autoimmune thyroiditis [2], through cytokine networks. EC produce various cytokines, such as interleukin 1 (IL-1), interleukin 6 (IL-6), colony stimulating factors and chemotactic factors [3, 4], and express adhesion molecules [5]. The expression of ICAM-1, a ligand of lymphocyte function-associated antigen 1 (LFA-1), on EC has been reported in inflammatory lesions and induced by inflammatory cytokines, such as IFNγ, IL-1 and tumor necrosis factor α (TNFα) [5, 6]. ICAM-1-positive EC can attract lymphocytes into the lesions via LFA-1 on the surface of lymphocytes [7] and can present antigen to T cells [8] and thus may lead to a specific autoimmune process. Furthermore, HLA-DR antigen, which is aberrantly expressed on the surface of target cells in autoimmune diseases [9], is also expressed on EC and induced by IFNγ [10]. Although the question of whether the aberrant expression of HLA-DR antigen on EC and autoimmune target cells is the primary or secondary phenomenon remains to be determined, the HLA-DR antigen expression may

accelerate the autoimmune reactions to EC and target cells.

On the other hand, nicotinamide, which is an inhibitor of poly(ADP ribose) synthetase, has been shown to inhibit the development of diabetes in non-obese diabetic mice [11] and in humans [12], and more recently we have reported that nicotinamide suppressed the expression of MHC class II antigens on normal mouse islet cells induced by synergistic effects of IFNγ and TNFα [13].

In the present study we demonstrated for the first time the inhibitory effects of nicotinamide on IFNγ-induced ICAM-1 and HLA-DR, but not HLA-A, B, C antigen expression on cultured human umbilical endothelial cells.

## 3. Materials and Methods

### 3.1. *Cell culture*

Human umbilical vein endothelial cells (EC) were obtained from Kurabou Industries Ltd., Neyagawa, Japan. EC suspensions were cultured in modified MCDB131 supplemented with 2% (v/v) heat-inactivated fetal bovine serum containing 10 ng/ml of epidermal growth factor, 1 μg/ml of hydrocortisone, 50 μg/ml of gentamycin and 0.25 μg/ml of amphotericin B in tissue culture dishes according to the industrial manuals.

### 3.2. *Detection of ICAM-1 and HLA antigens by flow cytometry*

To detect ICAM-1 and HLA antigens, cells were treated with 0.05% trypsin and 0.02% EDTA solution, and incubated with mouse monoclonal antibodies against ICAM-1 (anti-ICAM-1 antibody: Immunotech S.A., Marseille, France) at 1:20 dilution, HLA-A, B, C antigens (Dako-HLA-ABC: Dakopatts A/S, Glostrup, Denmark) at 1:40 dilution and HLA-DR (Orthomune OKDR: Ortho Diagnostic Systems, Raritan, NJ, U.S.A.) at 1:20 dilution for 30 min on ice. After washing with PBS the cells were incubated with FITC-conjugated anti-mouse IgG(H + L) rabbit IgG $F(ab')_2$ (Wako Pure Chemical Industries, Osaka, Japan) at 1:40 dilution for 30 min on ice. After the final wash, the cells were analyzed by flow cytometry (Ortho Cytron, Ortho Diagnostic Systems). The cells were counted for 120 s. As the first antibody, negative control mouse monoclonal antibody (MsIgG2a: Coulter Immunology, Tokyo, Japan) at 1:40 dilution was used as negative control. The live cell number was also counted.

### 3.3. *Induction of ICAM-1 and HLA antigen expression on EC*

Third or fourth-passaged EC were seeded at $5 \times 10^4$/well in 24-well plates, and 7 days after seeding, EC monolayers were incubated with recombinant human IFNγ (Mallinckrodt Inc., Kentucky, U.S.A.) for three days at 37 °C in 95% air/5% $CO_2$, and cells were analysed for ICAM-1 and HLA antigen expression.

### 3.4. *Effects of nicotinamide on IFNγ-induced ICAM-1 and HLA antigen expression on EC*

To investigate the effect of nicotinamide on ICAM-1 and HLA antigen expression on EC, EC were incubated with 200 U/ml of IFNγ in the presence of various concentrations of nicotinamide (Sigma; 1, 5, 10, 20 mM) for 3 days at 37 °C in 95% air/5% $CO_2$. Then again cells were analyzed for ICAM-1 and HLA antigen expression by flow cytometry. Cell numbers were also counted.

### 3.5. *Effects of nicotinamide on [$^3$H]thymidine incorporation into EC*

EC ($2 \times 10^4$/well) were cultured in triplicate in 96-well plates in 100 μl of culture medium. To test the effect of nicotinamide on the DNA synthesis of EC, the cells were cultured with various concentrations of nicotinamide for 72 h in the presence of 200 U/ml of IFNγ. Twenty-four hours before termination of the culture, 0.15 μCi (5.55 kBq) of methyl-[$^3$H]thymidine (DuPont/NEN Research Products, 247.9 GBq/mmol) was added to each well. The cells were washed and then harvested on glass fiber filter (Labo Science, Tokyo, Japan) using a semiautomatic cell harvester (Labo Mash, Labo Science, Tokyo, Japan). The radioactivity of each sample was determined in a liquid scintillation counter.

Statistical analysis was carried out using paired or unpaired Student's *t*-test.

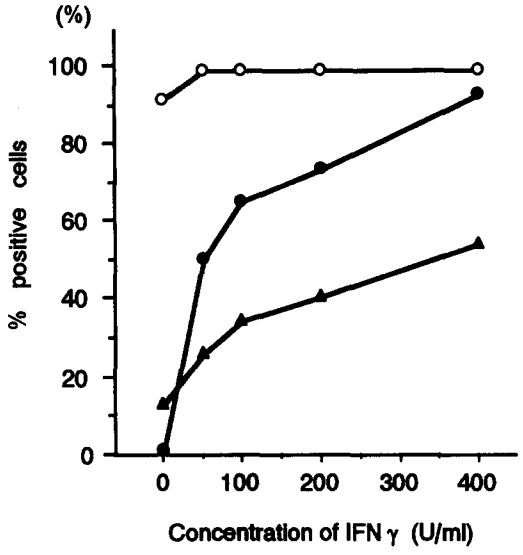


Fig. 1. Dose-response curves for ICAM-1, HLA-A, B, C and HLA-DR antigen expression on endothelial cells (EC). EC were exposed to increasing doses of IFNγ for 3 days. Cells were stained with monoclonal antibody ICAM-1 (▲), HLA-A, B, C (○) or HLA-DR (●) followed by FITC-conjugated anti-mouse IgG (H + L) rabbit IgG F(ab')$_2$ and analyzed by flow cytometry. Results are expressed as percent positive cells.

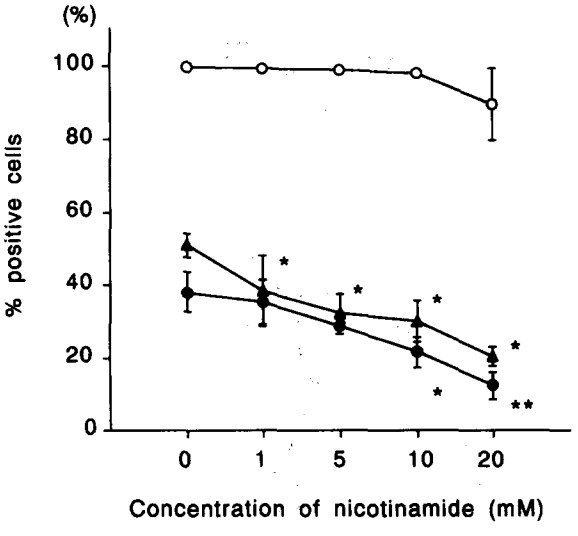


Fig. 2. Effect of nicotinamide on IFNγ-induced ICAM-1 (▲), HLA-A, B, C (○) and HLA-DR (●) antigen expression on EC. EC were treated with increasing doses of nicotinamide in the presence of IFNγ (200 U/ml) for 3 days. Results were expressed as mean (± SD) % positive cells of four individual experiments. $*P<0.05$; $**P<0.01$.

## 4. Results

### 4.1. *Augmentation of ICAM-1 and HLA antigen expression by IFN-γ*

Cultured human EC did not express HLA-DR antigens (0.9 – 1.1%). HLA-A, B, C antigens were detected in more than 90% of EC, and ICAM-1 were detected in 11.2 ~ 17.9% of EC. IFNγ induced dose-dependently ICAM-1, HLA-DR and HLA-A, B, C antigen expression on EC (Fig. 1). In the following experiments, ICAM-1 and HLA antigen expression was analyzed 3 days after the supplementation of 50 – 200 U/ml of IFNγ.

### 4.2. *Inhibitory effects of nicotinamide on ICAM-1 and HLA-DR antigen expression*

Nicotinamide inhibited in a dose-dependent manner both the ICAM-1 and HLA-DR antigen expression induced by 200 U/ml of IFNγ. The ratio of ICAM-1-positive cells in culture with nicotin-

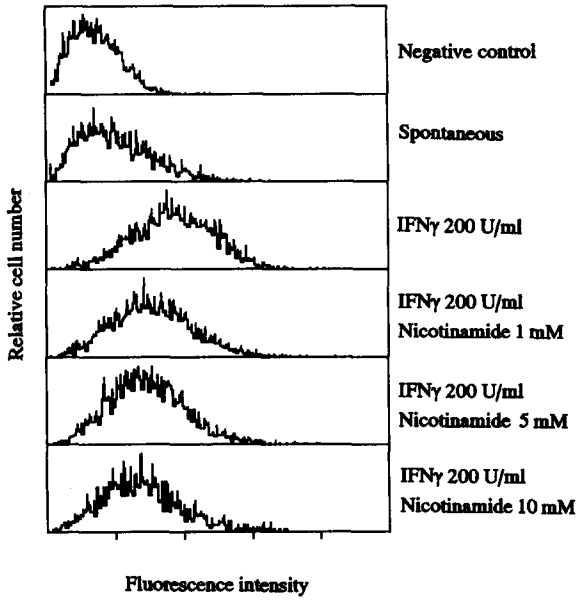


Fig. 3. Effect of nicotinamide on IFNγ-induced ICAM-1 expression on EC, assessed as immunofluorescence intensity in a representative experiment. EC were cultured for 3 days in medium alone or in the presence of 200 U/ml of recombinant human IFNγ and various concentrations of nicotinamide (0 – 10 mM). Cells were evaluated for ICAM-1 expression using monoclonal antibody ICAM-1 followed by FITC-conjugated anti-mouse IgG (H + L) rabbit IgG F(ab')$_2$ on flow cytometry.

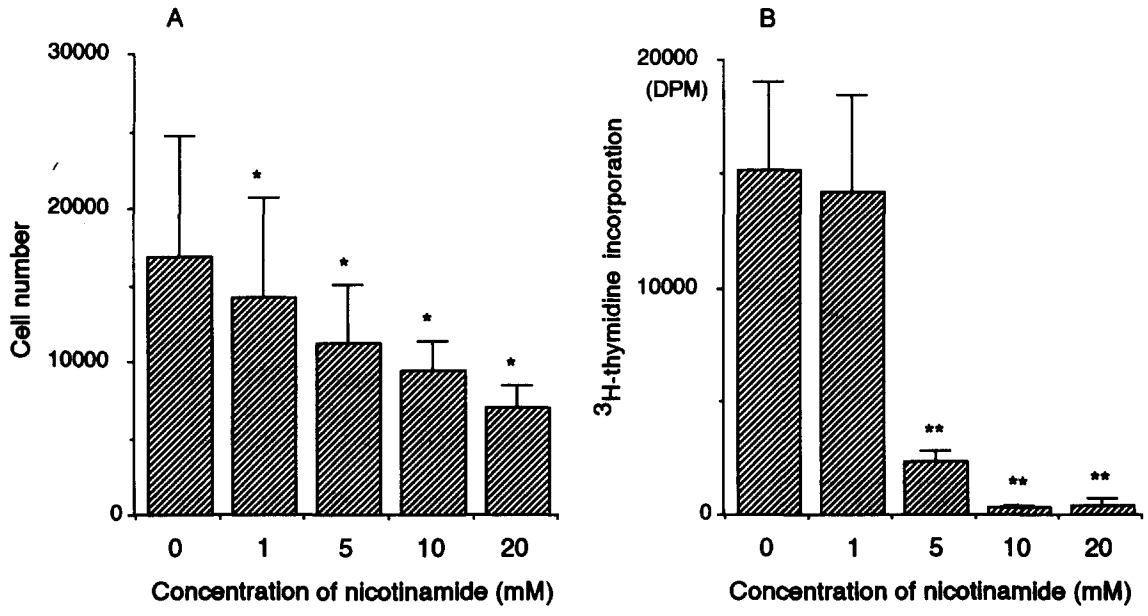


Fig. 4. Effect of nicotinamide on EC proliferation. See text for details. (A) Endothelial cell numbers at the estimation of ICAM-1 expression. Each point represents mean (±SD) of 5 individual experiments. (B) DNA synthesis in EC determined by [$^3$H]thymidine incorporation. Each point represents mean (±SD) DPM of triplicate values in a representative experiment. *$P<0.05$; **$P<0.01$.

amide was significantly lower: 32.1±5.3% at 5 mM, 30.1±5.9% at 10 mM, 20.4±2.7% at 20 mM, compared to that in the absence of nicotinamide (51.2±3.3%, $P<0.05$, Fig. 2). Mean channel of immunofluorescence intensity was also dose-dependently reduced by nicotinamide from 100.0 in the absence of nicotinamide to 81.5 at 1 mM, 77.2 at 5 mM, 76.3 at 10 mM of nicotinamide (Fig. 3). Nicotinamide also inhibited HLA-DR antigen expression on EC; 21.5±4.3% at 10 mM ($P<0.05$), 12.3±3.8% at 20 mM ($P<0.01$), compared to that without nicotinamide (38.2±5.7%; Fig. 2). Mean channel of immunofluorescence intensity was also dose-dependently reduced by nicotinamide from 66.9 in the absence of nicotinamide to 65.3 at 1 mM, 52.0 at 5 mM, 47.3 at 10 mM, 40.2 at 20 mM of nicotinamide. On the other hand, HLA-A, B, C antigen expression was not influenced by nicotinamide (Fig. 2). Experiments were repeated using 50 U/ml of IFNγ and similar results were obtained. ICAM-1 expression induced by 50 U/ml of IFNγ was reduced from 40.9% to 32.1% at 1 mM, 13.5% at 5 mM, 7.0% at 10 mM and 5.7% at 20 mM of nicotinamide.

### 4.3. *Effects of nicotinamide on EC proliferation*

The effects of nicotinamide on EC proliferation were determined by counting cell number of each culture and measurement of DNA synthesis assessed as [$^3$H]thymidine incorporation. Both cell number and [$^3$H]thymidine incorporation were dose-dependently suppressed by nicotinamide (Fig. 4).

## 5. Discussion

In the present study we demonstrated that nicotinamide inhibited the IFNγ-induced ICAM-1 and HLA-DR antigen expression on human EC. This was not observed in the case of IFNγ-induced HLA-A, B, C antigen expression. Nicotinamide, a potent inhibitor of poly(ADP ribose) synthetase, which is involved in DNA repair [14], cell differentiation [15], transformation [16], and transcription [17], has been shown to prevent alloxan- or streptozotocin-induced diabetes [18], and also autoimmune-associated diabetes in NOD mice [11] and humans [12]. Although the mechanism of the prevention of islet cell damage has not been determined, nicotinamide may prevent the depletion of

intracellular NAD, which is consumed as the substrate in ADP ribosylation. Moreover, recently we reported that nicotinamide inhibited the induction of MHC class II antigen expression on normal mouse islet cells by synergistic effects of IFNγ and tumor necrosis factor [13]. In the present study we have confirmed the inhibitory effect of nicotinamide on HLA-DR antigen expression using cultured human EC. Furthermore we demonstrated for the first time the inhibitory effect of nicotinamide on IFNγ-induced ICAM-1 expression on EC. However, it still remains to be clarified whether the inhibition of IFNγ-induced ICAM-1 and HLA-DR antigen expression on EC is related to the prevention of NAD depletion, free radical scavenging or other unknown mechanisms. The mechanism of the differential effects of nicotinamide on ICAM-1, HLA-DR and HLA-A, B, C antigen expression is also unclear. Experiments are currently in progress to elucidate these problems in our laboratory.

There are several lines of evidence that the expression of ICAM-1 and HLA-DR antigen on the surface of EC or autoimmune target cells, especially thyroid cells, may play an important role in the pathogenesis of autoimmune disorders [1, 2, 9, 19–21]. Indeed, Weetman et al. [19] reported that Graves' thyroid cells expressed ICAM-1 on the surface and ICAM-1 expression was induced by IFNγ, IL-1 and TNFα. Martin et al. [20] also reported the induction of ICAM-1 expression on cultured human thyroid cells and ICAM-1-mediated lymphocyte binding by IFNγ, which might result in autoimmune reactions such as antigen presentation and cytotoxicity [7]. In addition, Londei et al. [21] reported that HLA-DR-positive thyroid cells can present autoantigens to T cells and may augment the autoimmune process in the thyroid glands. Therefore, the suppression of ICAM-1 as well as HLA-DR antigen expression on EC by nicotinamide may reduce the inflammatory reactions associated with autoimmune disorders. Furthermore, nicotinamide suppressed the EC proliferation. As angiogenesis is common in the lesion of autoimmune disorders, the suppression of EC proliferation by nicotinamide may attenuate the inflammatory process.

In conclusion, nicotinamide inhibits the IFNγ-induced ICAM-1 and HLA-DR antigen expression on EC and EC proliferation. Further studies are indicated to elucidate whether suppressive effect of nicotinamide on ICAM-1 and HLA-DR expression is related to the prevention of the development of autoimmune disorders in vivo.

## References


[1] Kawakami, A., Eguchi, K., Migita, K., Nakao, H., Otsubo, T., Ueki, Y., Shimomura, C., Tezuka, H., Matsunaga, M., Ishimaru, T. and Nagataki, S. (1990) J. Rheumatol. 17, 430.

[2] Kabel, P. J., Voorbij, H. A. M., de Haan-Meulman, M., Pals, S. T. and Drexhage, H. A. (1989) J. Clin. Endocrinol. Metab. 68, 744.

[3] Miossec, P., Cavender, D. and Ziff, M. (1986) J. Immunol. 136, 2486.

[4] Mantovani, A. and Dejana, E. (1989) Immunol. Today 10, 370.

[5] Rothlein, R., Dustin, M. L., Marlin, S. D. and Springer, T. A. (1986) J. Immunol. 137, 1270.

[6] Dustin, M. L., Rothlein, R., Bhan, A. K., Dinarello, C. A. and Springer, T. A. (1986) J. Immunol. 137, 245.

[7] Makgoba, M. W., Sanders, M. E., Ginther, Luce, G. E., Dusstin, M. L., Springer, T. A., Clark, E. A., Mannoni, P. and Shaw, S. (1988) Nature 331, 86.

[8] Burger, D. R., Ford, D., Vetto, R. M., Hamblin, A., Goldstein, A., Hubbard, M. and Dumonde, D. C. (1981) Hum. Immunol. 3, 209.

[9] Hanafusa, T., Pujof-Borrel, R., Chiovato, L., Russell, R. C. G., Doniach, D. and Bottazzo, G. F. (1983) Lancet ii, 1111.

[10] Pober, J. S., Gimbrone, M. A., Cotran, R. S., Reiss, C. S., Burakoff, S. J., Fiers, W. and Ault, K. A. (1983) J. Exp. Med. 157, 1339.

[11] Yamada, K., Nonaka, K., Hanafusa, T., Miyazaki, A., Toyoshima, H. and Tarui, S. (1982) Diabetes 31, 749.

[12] Elliot, R. B. and Chase, H. P. (1991) Diabetologia 34, 362.

[13] Yamada, K., Miyajima, E. and Nonaka, K. (1990) Diabetes 39, 1125.

[14] Shall, S. (1984) Adv. Rad. Biol. 11, 1.

[15] Ohashi, Y., Ueda, K., Hayaishi, O., Ikai, K. and Niwa, O. (1984) Proc. Natl. Acad. Sci. USA 8, 7132.

[16] Juarez-Salinas, H., Sims, J. L. and Jacobson, M. K. (1979) Nature 282, 740.

[17] Taniguchi, T., Agemori, M., Kameshita, I., Nishikimi, M. and Shizuta, Y. (1982) J. Biol. Chem. 257, 4027.

[18] Uchigata, Y., Yamamoto, H., Kawamura, A. and Okamoto, H. (1982) J. Biol. Chem. 257, 6084.

[19] Weetman, A. P., Cohen, S., Makgoba, M. W. and Borysiewicz, L. K. (1989) J. Endocrinol. 122, 185.

[20] Martin, A., Huber, G. K. and Davies, T. F. (1990) Endocrinology 127, 651.

[21] Londei, M., Bottazzo, G. F. and Feldmann, M. (1985) Science 228, 85.