Albumin and Antioxidants Inhibit Serum-deprivation-induced Cell Adhesion in Hematopoietic Cells

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(Received May 16, 2008; Revised May 27, 2008 ; Accepted June 10, 2008)

Abstract – Previously, we identified albumin as an inhibitory factor in serum for cell adhesion of T cells such as human Jurkat T and primary cultured human T cells. In the present study, we found that other hematopoietic cell lines including U-937 human monocytes, THP-1 human monocytes, K-562 promyelocytic leukemia cells, and HL-60 human leukemia cells, also adhere to tissue culture flasks when serum is withdrawn, and albumin exerts an inhibitory effect on cell adhesion by those cells, implying that this inhibition is a common phenomenon in hematopoietic cells. Furthermore, we found that cell adhesion is inhibited by antioxidants such as (-)-epigallocatechin-3-gallate (EGCG), morin, and α -tocopherol. Our results suggest that albumin may inhibit basal cell adhesion of hematopoietic cells and that the oxidative balance in the plasma may be important for cell adhesion of hematopoietic cells *in vivo*.

Keywords : Monocytes, Leukocytes, Albumin, Adhesion, Hematopoietic cells, Oxidative signaling

Abbreviations - BSA, bovine serum albumin; FBS, fetal bovine serum; EGCG, (-)-epigallocatechin-3-gallate; morin, 3,5,7,2',4'-pentahydroxyflavone; ECM, extracellular matrix

INTRODUCTION

Hematopoietic cells including T cells and monocytes circulate in the blood. When they are activated by a variety of stimuli such as cytokines, they migrate to target tissues where immune responses occur (Dustin and de Fougereolles, 2001; Tseng and Dustin, 2002). The attachment and migration of activated cells are well characterized and the involvement of adhesion molecules such as ICAMs has been investigated extensively (Campanero *et al.*, 1994; Dustin and de Fougereolles, 2001; Tseng and Dustin, 2002). However, cell adhesion and migration of inactivated hematopoietic cells has been poorly investigated. In the previous study, we reported that bovine serum albumin (BSA), the

most studied and well-understood protein in plasma, inhibited the adhesion of T cells, such as the human Jurkat T cell line and primary cultured human T cells, probably independent of integrin-mediated signaling (Kim *et al.*, 2006). In the present study, we tested whether this effect of albumin is common in hematopoietic cells by using U-937 human monocytes, THP-1 monocytes, K-562 promyelocytic leukemia cells, and HL-60 human leukemia cells and also whether oxidative signaling is involved in cell adhesion by using various antioxidants such as (-)-epigallocatechin-3-gallate (EGCG), morin, and α -tocopherol, because oxidative signaling has been implicated in inflammation-mediated cell adhesion (Chen *et al.*, 2006; Sen and Roy, 2001).

METHODS AND MATERIALS

Materials and cell culture

Fetal bovine serum (FBS) was purchased from Hyclone

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Fig. 1. Inhibitory effect of albumin on cell adhesion of hematopoietic cells BSA concentration-dependence of cell adhesion in U-937 (A), THP-1 (B), K-562 (C), and HL-60 cells (D). Cells were incubated for 30 min in tissue culture dishes with 10% FBS (filled column) or without FBS and various concentrations of BSA (open column), and the number of adherent cells was calculated. Statistical significance: * $P < 0.05$, ** $P < 0.01$ vs. control without FBS and BSA.

(Logan, UT, USA) and bovine serum albumin (BSA, essentially fatty acid-free), EGCG, and α -tocopherol were from Sigma (St. Louis, MO, USA). Morin (3,5,7,2',4'-pentahydroxyflavone) was provided by the Aging Tissue Bank, Research Institute of Genetic Engineering (Pusan National University). Jurkat T cells, U-937 monocytes (CRL-1593.2), THP-1 monocytes (TIB-202), HL-60 human leukemia (CCL-240), and K-562 promyelocytic leukemia cells (CCL-243) were purchased from American Type Culture Collection (Manassas, VA, USA) and maintained in RPMI 1640 media, containing 10% (v/v) FBS, 100 units/ml penicillin, 50 μ g/ml streptomycin, 2 mM glutamine, and 1 mM sodium pyruvate at 37°C in a humidified 5% CO₂ incubator.

Cell adhesion

Cells (5×10^5 cells/mL) were seeded onto 48-well plates (BD Falcon, San Jose, CA, USA) for 30 min at 37°C. Media were discarded and non-adherent cells were removed by washing the wells twice with phosphate-buffered saline (PBS). Adherent cells were counted or stained with eosin Y. For eosin Y staining, cells that adhered to the wells were fixed overnight with 2% formaldehyde and stained with eosin Y for 30 min. Eosin Y was then extracted by the addition of a mixture of 1% of acetic acid and 50% ethanol, and

the absorbance was measured at 570 nm (Kim *et al.*, 2006).

Statistical analysis

The results are expressed as mean $\pm$ SE of the number of determinations indicated. Statistical significance of differences was determined by ANOVA. Significance was accepted when $P < 0.05$.

RESULTS

Inhibitory effect of albumin on adhesion of hematopoietic cells

Jurkat T cells or human primary T cells adhered to a tissue culture flask when 10% fetal bovine serum (FBS) was withdrawn (Kim *et al.*, 2006), and T cell adhesion was inhibited by bovine serum albumin (BSA) in FBS (Kim *et al.*, 2006). To test whether this effect of albumin is common in hematopoietic cells, we investigated the effects of FBS and albumin on cell adhesion in U-937 monocytes, THP-1 monocytes, K-562 promyelocytic leukemia cells, and HL-60 human leukemia cells. As shown in Fig. 1, we found that the cells adhered to a tissue culture flask when 10% FBS was withdrawn and that albumin inhibited the adhesion of all tested cell lines in a concentration-depen-

Fig. 2. Effects of FBS and BSA on cell adhesion of U-937 monocytes and C6 glioma cells U-937 monocytes (upper panels) or C6 glioma cells (lower panels) were incubated for 30 min for U-937 cells or 1 h for C6 cells in tissue culture dishes without FBS, with 10% FBS or with various concentrations of BSA in the culture media. Cells were photographed. Phase-contrast photographs $\times 400$.

dent manner (Fig. 1). Significant inhibition of adhesion was observed at BSA concentrations over 0.5% or 1% depending on cell type. To test whether the effect of albumin is specific to hematopoietic cells, we investigated the effects of FBS and albumin on cell adhesion in rat C6 glioma and HCT116 human colon cancer cells. As shown in Fig. 2, we found that adhesion of C6 cells was not influenced by FBS deprivation. BSA disrupted cell adhesion of C6 cells in a concentration-dependent manner (Fig. 2). However, higher BSA concentration (5%) was necessary to inhibit the cell adhesion of C6 glioma cells than that required to inhibit U-937 monocytes. Also, severe aggregation was observed in C6 glioma cells (Fig. 2). Similar results of cell adhesion in C6 glioma cells were also observed in HCT116 colon cancer cells (data not shown).

This implies that high concentrations of BSA blocked interaction between C6 glioma cells and ECM (extracellular matrix); therefore cells started to aggregate because integrin-mediated cell adhesion to tissue culture plates was not possible due to BSA blocking. The inhibitory effect of BSA, however, was more clearly observed at very low concentrations in hematopoietic cells as shown in Fig. 2 (Fig. 2). Therefore, the inhibitory effect of BSA on cell adhesion is considered to be specific to hematopoietic cells, because the physiological concentration of albumin in plasma is 0.6 mM and adherent cells are rarely in the blood stream (Quinlan *et al.*, 2005).

Effect of antioxidants on cell adhesion

Next, we tested the effects of various antioxidants on

Fig. 3. Effect of EGCG on cell adhesion of hematopoietic cells EGCG concentration-dependence of cell adhesion in U-937 (A), THP-1 (B), K-562 (C), and HL-60 cells (D). Cells were incubated for 30 min in tissue culture dishes with 10% FBS (filled column) or without FBS (open column) in the presence of various concentrations of EGCG, and the number of adherent cells was calculated. Statistical significance: * $P<0.05$, ** $P<0.01$ vs. control without FBS and EGCG.

Fig. 4. Effect of morin on cell adhesion of hematopoietic cells Morin concentration-dependence of cell adhesion in U-937 (A), THP-1 (B), K-562 (C), and HL-60 cells (D). Cells were incubated for 30 min in tissue culture dishes with 10% FBS (filled column) or without FBS (open column) in the presence of various concentrations of morin, and the number of adherent cells was calculated. Statistical significance: * $P < 0.05$, ** $P < 0.01$ vs. control without FBS and morin.

cell adhesion in the four hematopoietic cell lines, because oxidative signaling has been implicated in inflammation-mediated cell adhesion (Chen *et al.*, 2006; Sen and Roy, 2001). As shown in Fig. 3, EGCG, a polyphenolic antioxidant catechin from green tea, significantly inhibited the cell adhesion in a concentration-dependent manner in all

tested cell lines. The effect was most potent in HL-60 leukemic cells, where complete inhibition was observed at 3 μM EGCG. However, for U-937, THP-1, and K-562 cells, 100 ~ 300 μM EGCG was necessary for complete inhibition. Morin, a flavone antioxidant from the Moraceae family, which includes mulberry figs, also inhibited the cell

Fig. 5. Effect of α -tocopherol on cell adhesion of hematopoietic cells α -Tocopherol concentration-dependence of cell adhesion in U-937 (A), THP-1 (B), K-562 (C), and HL-60 cells (D). Cells were incubated for 30 min in tissue culture dishes with 10% FBS (filled column) or without FBS (open column) in the presence of various concentrations of α -tocopherol, and the number of adherent cells was calculated. Statistical significance: * $P < 0.05$, ** $P < 0.01$ vs. control without FBS and without α -tocopherol.

adhesion of all cell types (Fig. 4); U-937 monocytes were more sensitive to morin than the other cell lines. In order to confirm whether oxidative signaling is involved in cell adhesion, we tested the effect of α -tocopherol, the lipid-soluble antioxidant vitamin, in cell adhesion. As shown in Fig. 5, α -tocopherol significantly inhibited the adhesion of the four tested cell lines in a concentration-dependent manner.

DISCUSSION

To expand on our previous findings, we tested four hematopoietic cells and found that, without serum, all cell types adhere to culture flasks and that the adhesion is inhibited by albumin. To generalize our previous findings: 1) Hematopoietic cells adhere to culture flasks in serum-free conditions, 2) Albumin in serum is the main inhibitory factor for maintaining the non-adherence of hematopoietic cells. Although *in vivo* experiments are necessary to support the physiological significance of our findings, it is obvious that albumin in serum blocks basal cell adhesion of hematopoietic cells *in vitro*. The cell adhesion of hematopoietic cells to culture flasks differed from that of C6 glioma cells to culture flasks. FBS deprivation did not influence the C6 glioma cell adhesion, in contrast to the dependence of hematopoietic cell adhesion on FBS deprivation. Furthermore, BSA did not influence C6 glioma cell adhesion at low concentrations (0.1 and 1%), excluding the non-specific blocking effect of BSA which caused the inhibition in hematopoietic cells. We tested the effects of various antioxidants and found that cell adhesion was abolished by treatment with antioxidants such as EGCG, morin, and α -tocopherol. These three chemicals have different chemical structures, but have an antioxidant function in common. Furthermore, two other antioxidants, resveratrol and N-acetyl-L-cysteine also showed inhibitory effects on cell adhesion in these four cell lines (data not shown). Therefore, oxidative signaling is thought to be important for the adhesion process. In the literature, some other mechanisms of action have been reported for those antioxidants. The action of α -tocopherol is thought to be mediated by its antioxidant properties, mainly by protecting the polyunsaturated fatty acids in the plasma membrane and the membrane qualities (fluidity, phase separation, and lipid domains) (Traber and Atkinson, 2007), although an unknown target of α -tocopherol has also been suggested (Azzi, 2007). In addition to its antioxidant function, morin has been shown to exhibit anti-inflammatory and antitumor activities through suppression of NF- κ B activation (Manna et al., 2007). EGCG, a polyphenolic compound

from green tea, has antioxidative, antithrombogenic, anti-allergic, and anti-inflammatory properties. Its inhibitory action on cell adhesion has previously been studied in the prevention of atherosclerosis and cancer metastasis (Hung et al., 2005; Ludwig et al., 2004; Zhang et al., 2000). The interaction of EGCG with fibronectin and laminin was also suggested as a mechanism (Sazuka et al., 1998; Suzuki and Isemura, 2001). Binding of EGCG to CD11b, a member of α -chain integrin in CD8⁺ T cells, was shown to attenuate adhesion and migration of CD8⁺ T cells (Kawai et al., 2004). In a later study, however, multiple mechanisms were suggested to explain the EGCG-induced inhibition of fibroblast adhesion and migration, including decreased expression and affinity of integrin α 2 β 1, decrease of intracellular H₂O₂, focal adhesion kinase phosphorylation, actin cytoskeleton reorganization, and inhibition of matrix metalloproteinase activity and expression (Hung et al., 2005). The differential sensitivity of these cell lines to each antioxidant might imply that another mode of action is in effect for these three antioxidants, other than the antioxidant function, such as in the multiple mechanisms of action of EGCG (Hung et al., 2005). However, the antioxidative function of these three chemicals is thought to be the main cause of the inhibition of cell adhesion. Inappropriate or abnormal sequestration of leukocytes at specific sites is a central component in the development of a variety of autoimmune diseases and pathologic inflammatory disorders (Collins et al., 1994; Dailey, 1998).

Cell adhesion molecule expression and adhesive properties of cells are greatly modified by several conditions involving redox imbalances (Sen and Roy, 2001). Oxidants such as H₂O₂ are known to induce adherence of leukocytes to endothelial cells (Roy et al., 1999; Sellak et al., 1994). Several antioxidants down-regulate inducible cell adhesion molecule expression as well as cell-cell adhesion (Sen and Roy, 2001). Agonist (interleukin-1, thrombin, or PMA)-induced monocytic adhesion to HUVEC, but not basal adhesion, was down-regulated by α -tocopherol (Faruqi et al., 1994). Quercetine, a potent flavonoid antioxidant, suppressed agonist-induced ICAM-1 protein and gene expression (Kobuchi et al., 1999). Inflammatory cytokine-stimulated endothelial adhesion to human monocytic cells could be suppressed by drugs with antioxidant activity that attenuated intracellular ROS formation, redox-sensitive transcription factor activation, and VCAM-1 and ICAM-1 expression (Chen et al., 2006). Another interpretation of our results is that the cells were not activated by any stimulants. Adhesion and migration of hematopoietic cells to endothelial cells are evoked by

stimuli such as inflammatory cytokines. However, in our experiment, the withdrawal of serum made cells adhere and the presence of albumin inhibited cell adhesion. Therefore, our results may imply the significance of hematopoietic cell basal adhesion and its modulation by albumin and oxidative balance in plasma. In summary, in the present study, we found that albumin-induced inhibition of cell adhesion is common in hematopoietic cells and that antioxidants inhibit the basal cell adhesion of hematopoietic cells *in vitro*. These results imply that albumin and the redox status in plasma may modulate basal adhesion of hematopoietic cells *in vivo*.

ACKNOWLEDGEMENT

This work was supported by the Grand-in-aid for 2007 Korea-Japan Joint Research Project from Korea Science and Engineering Foundation (F01-2007-000-10005-0).

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