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Effects of Mixed Leukocyte Reaction, Hydrocortisone and Cyclosporine on Expression of Leukocyte Adhesion Molecules by Endothelial and Mesangial Cells

Chun-Gyoo Ihm, M.D.[1], Seong-Pyo Hong, M.D.[1], Jae-Kyung Park, M.S.[1], Tae-Won Lee, M.D.[1], Byoung-Soo Cho, M.D.[2], Mun-Ho Yang, M.D.[3], Myung-Jae Kim, M.D.[1]

Department of Internal Medicine [1], Department of Pediatrics [2], Department of Pathology [3], College of Medicine, Kyung Hee University, Seoul, Korea

We investigated the effects of mixed leukocyte reaction (MLR), hydrocortisone (HC) and cyclosporine A (CsA) on the expression of leukocyte adhesion molecules on the mesangial (MC) and endothelial cells (EnC). Cell surface enzyme immunoassay showed that INFγ, IL-1β, or TNF α stimulated expression of ICAM-1, or VCAM-1 on MC after 24 hours. Flow cytometric analysis demonstrated that MLR supernatant induced a marked increase in mean fluorescence of or % of cells highly expressing intercellular adhesion molecule (ICAM)-1 or vascular cell adhesion molecule (VCAM)-1 on both cells after 24 hours ($p<0.001$). HC treatment(300 ng/ml) during MLR effectively inhibited MLR-induced upregulation of ICAM-1 and VCAM-1 on both cells ($p<0.005$). When MLR supernatant with HC was added to adhesion molecule assay, there was no inhibitory effect of HC on VCAM-1. CsA treatment (500 ng/ml) during MLR caused a modest decrease in upregulation of VCAM-1 on EnC ($p<0.05$), but had no effects on ICAM-1 on both cells. CsA directly decreased expression of VCAM-1 on MC. In conclusion, alloreactive lymphocytes and monocytes upregulate the expression of VCAM-1 and ICAM-1 on target cells probably by the mediation of cytokines. HC effectively prevents MLR-induced upregulation of VCAM-1 and ICAM-1. CsA does not increase the expression of VCAM-1 and ICAM-1.

Key Words : *Alloreactive cells, ICAM-1, VCAM-1, Hydrocortisone, Cyclosporine*

INTRODUCTION

Mixed leukocyte reaction (MLR) is an in vitro correlate of graft rejection (Oppenheim et al., 1984), and alloreactive lymphocytes and monocytes secrete the proinflammatory cytokines (Thomas et al., 1992). Glucocorticoids inhibit the production of cytokines such as interferon (INF)γ or interleukin (IL)-2 by activated T cells (Arya et al., 1984 ; Paliogianni et al., 1993). Cyclosporine A (CsA) also inhibits the production of IL-2 and T cell activation, and hence has secondary effects on many types of cells (Bunjes et al., 1981 ; Reem et al., 1983). On the other hand, CsA is known to cause the nephrotoxicity such as vasculopathy or glomerulosclerosis (Zoya et al., 1986 ; Myers et al., 1988 ; Kopp and Klotman, 1990).

Address for correspondence : *Chun-Gyoo Ihm, M.D., Division of Nephrology, Department of Medicine, Kyung Hee University Medical Center. #1 Hoikidong, Dongdaemunku, Seoul 130-702, Korea.*
Tel : 82-2-958-8182, Fax : 82-2-968-1848.

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Leukocyte adhesion molecules, such as intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1, are shown to play an important role in immune or inflammatory reactions, and can be regulated by cytokines (Brennan et al., 1990 ; Graber et al., 1990 ; Wuthrich, 1992 ; Brady, 1994). It was reported that a marked increase in the expression of VCAM-1 or ICAM-1 was noticed by the renal allograft during rejection (Briscoe et al., 1992 ; Alpers et al., 1993 ; Gibbs et al., 1993 ; Mamppaso et al., 1993 ; Hill et al., 1995). It's interesting to determine whether immunosuppressive agents can modulate their expression.

Therefore, to investigate the interactions between alloreactive lymphocytes and monocytes, and target cells, we studied the effect of MLR on expression of VCAM-1 and ICAM-1 by mesangial (MC) and endothelial cells (EnC). We examined whether hydrocortisone (HC) or CsA can modulate their expression induced by MLR. This study also sought to determine whether CsA directly affects the expression, in relation to CsA nephrotoxicity.

MATERIALS AND METHODS

Materials

Human IL-1β, tumor necrosis factor (TNF)α, and INFγ were purchased from Genzyme (Cambridge, MA, USA). Human TGF β1 was obtained from R&D Systems (Minneapolis, Minnesota, USA). Monoclonal antibodies to ICAM-1 and VCAM-1 were purchased from Serotec (Oxford, England). Alkaline phosphatase labeled goat anti-mouse IgG was from GIBCO/BRL (Gaithersburg, Maryland, USA). Fluorescein-conjugated goat anti-mouse IgG was from Becton-Dickinson (Mountain View, CA, USA) and Ficoll-Paque from Pharmacia LKB Biotechnology AB (Uppsala, Sweden). HC and dimethyl sulfoxide (DMSO) were obtained from Sigma chemical (St. Louis, MO, USA), and CsA was donated by Sandoz (Basel, Swiss).

Experimental design

Peripheral blood mononuclear cells were prepared from heparinized blood taken from normal volunteers by Ficoll-Paque density gradient centrifugation and were adjusted to 1 ml aliquots of 5×10^6 cells. Two-way MLR was performed with or without CsA or HC for 5 days. CsA was prepared by adding 0.1% DMSO. MLR supernatant was collected by centrifugation. After adding 25% MLR supernatant, cytokines or CsA to MC or EnC, the expression of VCAM-1 and ICAM-1 was examined by using cell surface enzyme immunoassays (EIA) or flow cytometry.

Human MC culture

Collagenase treated glomeruli were plated on culture dishes in DMEM media containing 17% heat-inactivated fetal bovine serum. Near confluent cells in the third to fourth passage were used in these studies. Cells displayed the typical spindle or stellate morphology. Immunofluorescent staining of the cells was negative for antibodies to common leukocyte antigen and factor VIII, and positive for antibody to fibronectin. The cells were capable of growth in D-valine substituted medium (Border et al., 1990).

Human EnC culture

Human umbilical vein EnC were harvested enzymatically with 0.1% collagenase as described (Jaffe et al., 1973) and maintained in medium 199, containing Herpes (25mM), heparin (1%), EnC growth factor (50 ug/ml), L-glutamine (1%), antibiotics, and 10% fetal calf serum. Once grown to confluence, the cells were replated on fibronectin. The cells isolated by these techniques form a confluent monolayer of polygonal cells and express von Willebrand factor by immunofluorescent study.

Cell surface EIA of adhesion molecules

1×10^4/well MC were transferred to each well of 96-well plate and rested for 48 to 72 hours in serum-free medium. The expression of ICAM-1 and VCAM-1 was measured after 24 hours. Each plate was fixed with 1% paraformaldehyde for 15 min at room temperature and washed. After blocking unbound region with 200 μl of 2% bovine serum albumin, 100 μl of antibodies to ICAM-1 or VCAM-1 were added. Controls included cells without primary antibody or stained with irrelevant isotype matched antibody. Then alkaline phosphatase conjugated goat anti-mouse antibodies were added and cells were incubated for 1 hour. After adding p-nitrophenyl phosphate, reaction was stopped with 3 N NaOH. The optical density (OD) was read at 405 nm (Warner et al., 1989).

Flow cytometric analysis of adhesion molecules

Human MC and EnC were trypsinized lightly, washed with phosphate-buffered saline, and incubated with specific primary antibody for 30 min at 4℃. Controls included cells without primary antibody or stained with irrelevant isotype matched antibody. After further wash-

ing, the cells were incubated with FITC-conjugated secondary antibody for 30 min at 4℃. Cells were washed and fixed with 1% paraformaldehyde. Cells were passed through a FACScan analyzer (Becton-Dickinson Immunocytometry Systems, Mountain View, CA) (Brennan et al., 1990).

Statistics

Results are expressed as the mean±SD. Comparisons among groups were made by analysis of variance. Comparisons between groups were made using the Student's paired t-test.

RESULTS

Cytokines and leukocyte adhesion molecules

Cell surface EIA showed that INF γ (200 unit/ml), IL-1 β (1 ng/ml), or TNF α (1 ng/ml) stimulated expression of VCAM-1 and ICAM-1 on MC by 25-135%, compared to media ($p<0.05$).

MLR-induced expression of VCAM-1 and ICAM-1

Flow cytometric analysis on MC or EnC demonstrated that MLR supernatant markedly enhanced the mean fluorescence of VCAM-1 on EnC by 262% (media ; 13.1±0.4 vs MLR ; 47.5±0.8) and MC by 235% (media ; 13.4±0.5 vs MLR ; 45.1±0.5), and that of ICAM-1 on EnC by 481% (media ; 49.0±4.0 vs MLR ; 99.1±3.8) and MC by 840% (media ; 10.5±0.7 vs MLR ; 98.8±2.5) after 24 hours ($p<0.001$) (Fig. 1, 2, 3). MLR supernatant also enhanced % of cells highly expressing ICAM-1 or VCAM-1 by 102-836% (not shown).

Effects of HC and CsA on MLR-induced expression of VCAM-1

HC treatment (300 ng/ml) during MLR effectively inhibited the MLR-induced upregulation on MC (by 43%) and EnC (by 49%) ($p<0.005$) (Fig. 2). When MLR supernatant with HC was added to adhesion molecule assay, there was no inhibitory effect of HC. The supernatant obtained from MLR with CsA (500 ng/ml) added to MLR caused a modest reduction of MLR- induced upregulation of VCAM-1 on EnC by 19% ($p< 0.05$). When we tested whether CsA causes the upregulation of adhesion molecules, CsA directly decreased the basal VCAM-1 expression on MC by 18% ($p<0.05$). DMSO (0.1%) had no effect on the expression.

Effects of HC and CsA on MLR-induced expression of ICAM-1

HC treatment during MLR effectively inhibited MLR-induced upregulation of ICAM-1 on MC (by 38%) and EnC (by 24%) ($p<0.005$) (Fig. 3). However, CsA treatment during MLR had no effects on MLR-induced upregulation of ICAM-1 on both cells.

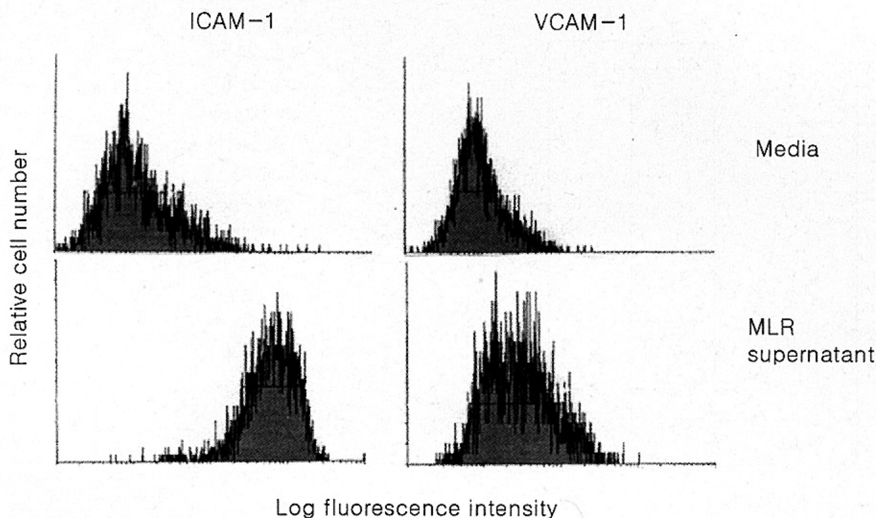

Fig. 1. Flow cytometric anaysis shows the effect of MLR supernatant on the expression of ICAM-1 and VCAM-1 on MC. Confluent monolayers of human MC were incubated for 24 hours with media alone or 25% MLR supernatant. Controls included cells without primary antibody or stained with irrelevant isotype matched antibody.

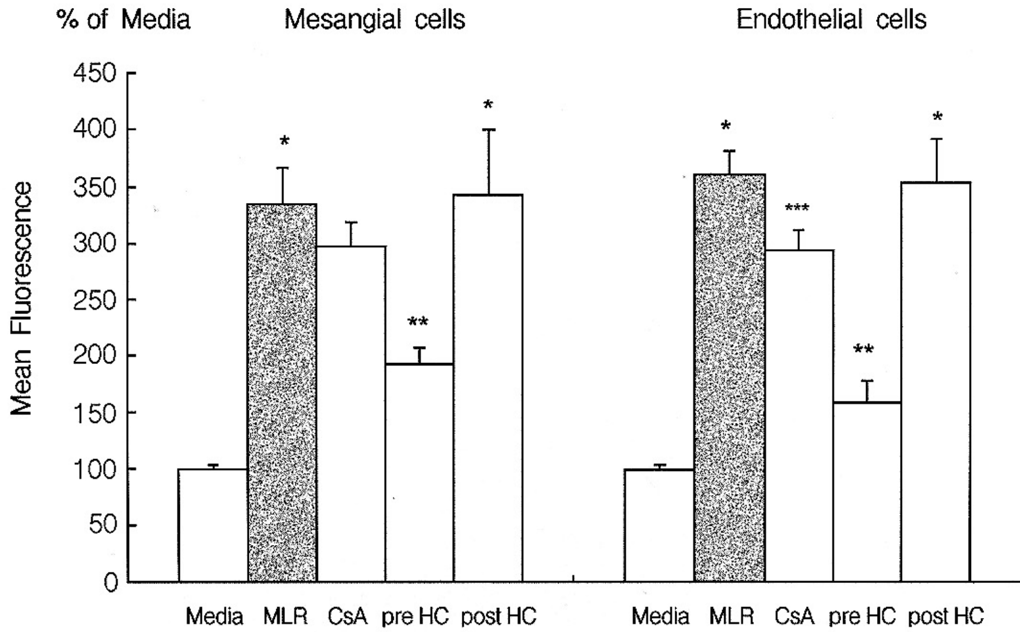

Fig. 2. Effect of CsA and HC on MLR-induced expression of VCAM-1. MLR supernatant markedly increased the mean fluorescence of VCAM-1 expression on both cells (*$p<0.001$). HC treatment (300 ng/ml) during MLR (pre HC) inhibited the MLR-induced upregulation (**$p<0.005$). When MLR supernatant with HC was added to adhesion molecule assay (post HC), however, there was no effect of HC. The supernatant obtained from MLR with CsA (500 ng/ml) added to MLR caused a modest decrease in MLR-induced upregulation of VCAM-1 on EnC (***$p<0.05$).

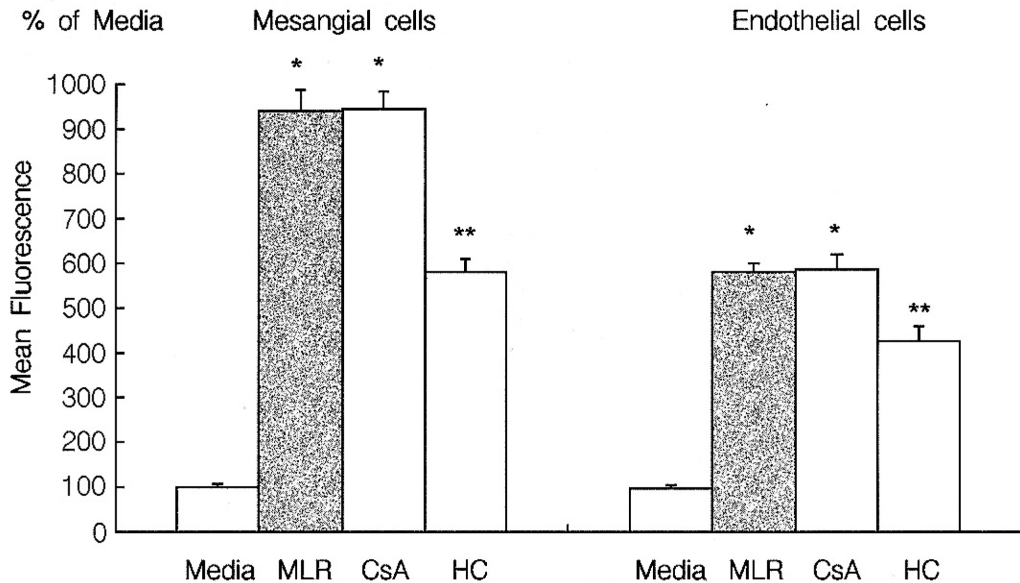

Fig. 3. Effect of CsA and HC on MLR-induced expression of ICAM-1. MLR supernatnat markedly increased the mean fluorescence of ICAM-1 expression (*$p<0.001$). HC treatment (300 ng/ml) during MLR inhibited the MLR-induced upregulation of ICAM-1 on both cells (**$p<0.005$). There was no inhibitory effect of CsA (500 ng/ml) on that of ICAM-1.

DISCUSSION

ICAM-1 and VCAM-1 are members of the immunoglobulin superfamily and are also expressed on EnC and MC (Wuthrich,1992 ; Brady,1994). Our study showed that expression of ICAM-1 and VCAM-1 on MC was increased by INF γ, IL-1 β, or TNF α on MC after 24 hours, as already reported (Brennan et al.,1990 ; Graber et al.,1990). Furthermore, it's not surprising that the expression of ICAM-1 and VCAM-1 on MC or EnC was markedly enhanced by MLR supernatant which was produced by alloreactive lymphocytes and monocytes. Therefore, various cytokines, such as INF γ, may be the main candidates in MLR supernatant for the upregulation of VCAM-1 and ICAM-1(Thomas et al., 1992), although experiments using cytokine blocking antibody need to be done. Our results are supported by other in vitro study, showing that CD4+ T cells directly caused the increase in the expression of ICAM-1 and VCAM-1 on EnC (Damle et al., 1991). This upregulation may facilitate attachment of leukocytes to the target cells in vascular or renal injury. It was reported that a marked increase in the expression of VCAM-1 or ICAM-1 was noticed by the renal vasculature, as well as proximal tubules, during graft rejection (Briscoe et al., 1992 ; Alpers et al., 1993 ; Gibbs et al., 1993 ; Mamppaso et al., 1993 ; Hill et al., 1995).

It was observed in this study that HC treatment during MLR effectively inhibited the MLR-induced upregulation of VCAM-1 and ICAM-1 on MC or EnC. Interestingly, however, there was no inhibitory effect of HC, when MLR supernatant with HC was added to adhesion molecule assay. This indicates that HC prevents the expression of VCAM-1 and ICAM-1 on target cells maybe by inhibiting the release of cytokines during MLR, but cannot inhibit the expression stimulated by already released cytokines. This is similar to the previous observation that dexamethasone had no effect on the basal or cytokine-induced expression of ICAM-1 and VCAM-1 on renal tubular cells (Wuthrich and Sekar, 1993). Others showed that glucocorticoids didn't inhibit TNFα-induced upregulated expression of ICAM-1 on EnC, while inhibited the endotoxin-induced expression through glucocorticoid receptor (Cronstein et al., 1992).

Our results showed that CsA treatment during MLR caused a modest decrease in MLR-induced expression of VCAM-1 on EnC, but had no effect on that of ICAM-1. Also CsA decreased the basal expression of VCAM-1 on MC. This decrease in VCAM-1 expression may be direct effect of CsA, inhibition of cytokine production by CsA (Bunjes et al., 1981 ; Reem et al., 1983), or be related to a decrease of MC proliferation caused by CsA (Radeke et al., 1991), but the precise mechanism remains uncertain in the present study. Other in vitro studies showed no direct effect of CsA on VCAM-1 or ICAM-1 expression by Langerhans cells or renal tubular cells (Teunissen et al.,1991 ; Wuthrich and Sekar,1993). It was also reported that CsA inhibited T cell activation but had no effect on the ability of T cell to upregulate endothelial VCAM-1 or ICAM-1 (Damle et al., 1991). These in vitro results can be related to the findings in CsA nephrotoxicity. A weak staining pattern for VCAM-1 of endothelium was observed in CsA nephrotoxicity, in contrast to a strong reactivity in rejection (Gibbs et al., 1993 ; Mamppaso et al., 1993). Thus it was suggested that the differential expression patterns could provide valuable criteria for the diagnosis of allograft dysfunction. On the other hand, there remain controversies about the expression of ICAM-1 in CsA nephrotoxicity (Gibbs et al., 1993 ; Mamppaso et al., 1993). Recently it was reported that CsA nephrotoxicity was preceded by a significant macrophage influx (Young et al., 1995). Taken together, it's possible that other macrophage adhesion molecules than VCAM-1 mediate the adhesion of macrophage in CsA nephrotoxicity.

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