Biochemical Pharmacology, Vol 46, No 8, pp 1349–1353, 1993
Printed in Great Britain

0006-2952/93 $6.00 + 0 00

EFFECT OF DEXAMETHASONE, 6-MERCAPTOPURINE AND CYCLOSPORINE A ON INTERCELLULAR ADHESION MOLECULE-1 AND VASCULAR CELL ADHESION MOLECULE-1 EXPRESSION

RUDOLF P. WUTHRICH* and PADMINI SEKAR

Division of Nephrology and Nephrology Research and Training Center, Department of Medicine, University of Alabama at Birmingham, UAB Station, Birmingham, AL 35294, U.S.A.

(*Received* 5 *February* 1993; *accepted* 12 *July* 1993)

Abstract—The expression of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) is increased in immune-mediated diseases. These adhesion molecules promote the adherence of inflammatory cells to parenchymal target cells. We have examined the effect of immunosuppressants on the expression of ICAM-1 and VCAM-1 in a renal tubular epithelial cell line. We found that dexamethasone (4–40 μg/mL), the azathioprine metabolite 6-mercaptopurine (10–100 μg/mL) and cyclosporine A (0.1–1 μg/mL) have no effect on the basal and the tumor necrosis factor-α- or interleukin-1-stimulated expression of these adhesion molecules. We conclude that these immunosuppressants do not directly influence the expression of ICAM-1 and VCAM-1 by renal tubular epithelium.

Intercellular adhesion molecule-1 (ICAM-1†) and vascular cell adhesion molecule-1 (VCAM-1) are glycosylated cell surface molecules which are expressed by vascular endothelial cells and by non-vascular cells such as the renal tubular epithelium [1, 2]. Through interaction with specific counter-receptors on leukocytes these adhesion molecules promote adherence of infiltrating T cells and monocytes to target cells [3–5]. ICAM-1 and VCAM-1 are overexpressed in various inflammatory diseases, for example in kidney allograft rejection and lupus nephropathy [6–10], presumably in response to locally released cytokines [tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1) and interferon-γ (IFN-γ)].

Upregulation of adhesion molecules is a mechanism whereby parenchymal cells become more adhesive for inflammatory cells [2, 3]. Thus, T cell and monocyte adhesion to renal tubular cells through ICAM-1/LFA-1 (leukocyte function-associated antigen-1) and VCAM-1/VLA-4 (very late antigen-4) interactions is promoted by increased ICAM-1 and VCAM-1 expression in the kidney. Inhibition of ICAM-1 and VCAM-1 expression in response to cytokines might therefore be a useful therapeutic goal to interrupt the interaction of T cells and monocytes with parenchymal cells in renal allograft rejection and other immunologically mediated kidney diseases. The purpose of the present study was thus to determine whether commonly used immunosuppressants have a direct inhibitory effect on renal tubular ICAM-1 and VCAM-1 expression.

* Corresponding author: Dr Rudolf P. Wuthrich, Medical Clinic B, Kantonsspital, 9007 St Gallen, Switzerland. Tel. (41) 71 26 11 11; FAX (41) 71 26 28 77.

† Abbreviations: AZA, azathioprine; CSA, cyclosporine A; DEX, dexamethasone; 6-MP, 6-mercaptopurine; ICAM-1, intercellular adhesion molecule-1; IFN-γ, interferon-γ; IL-1, interleukin-1; TEC, tubular epithelial cells; TNF-α, tumor necrosis factor-α; VCAM-1, vascular cell adhesion molecule-1.

MATERIALS AND METHODS

Materials. Tissue culture reagents were from Gibco (Grand Island, NY). Dexamethasone (DEX) and 6-mercaptopurine (6-MP) were from the Sigma

Table 1. Inhibition of anti-CD3-stimulated splenocyte proliferation

Drug	Concn (μg/mL)	anti-CD3	cpm
—	—	−	613 ± 58
—	—	+	210291 ± 8682
DEX	40	+	3755 ± 424
DEX	4	+	11093 ± 267
DEX	0.4	+	13562 ± 2258
AZA	100	+	299 ± 42
AZA	10	+	16830 ± 1108
AZA	1	+	120317 ± 10633
6-MP	100	+	2955 ± 18
6-MP	10	+	22580 ± 1489
6-MP	1	+	61328 ± 3083
CSA	1	+	409 ± 21
CSA	0.1	+	15735 ± 3290
CSA	0.01	+	75793 ± 12001

Splenocytes (6×10^5/well) were stimulated with anti-CD3 mAb 145-2C11 for 48 hr in 96-well plates in the presence of various drugs. [^{3}H]Thymidine incorporation was then measured as an index of proliferation.

Data represent means ± SE (N = 3).

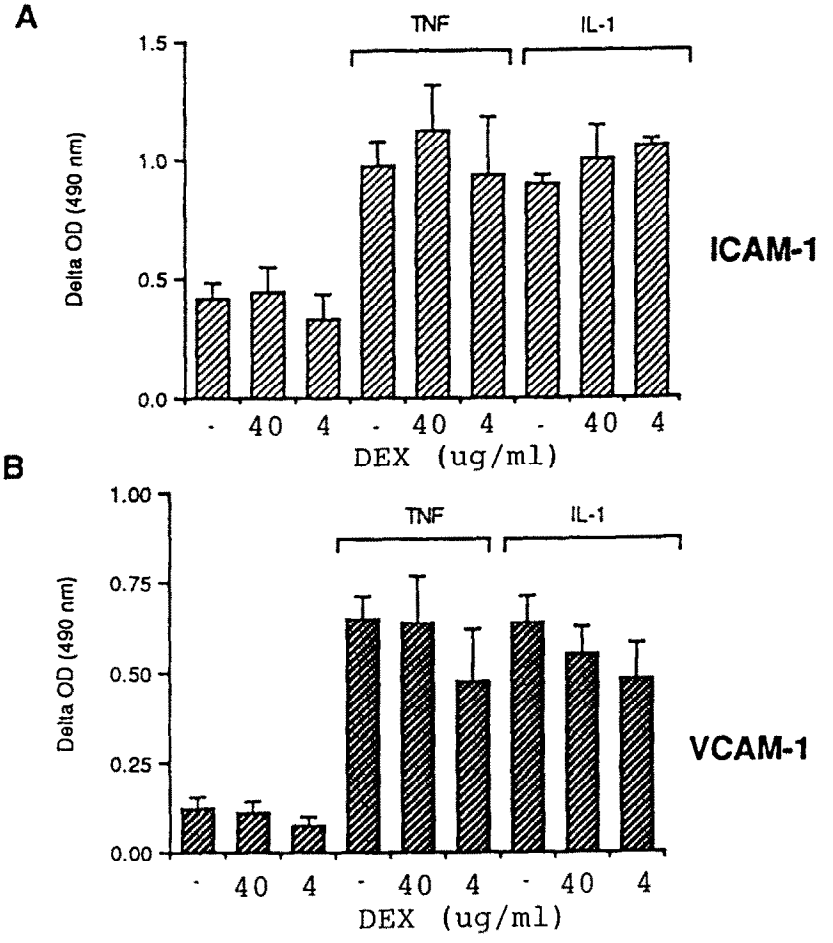

Fig. 1. Effect of DEX on TNF-α- and IL-1-stimulated ICAM-1 (A) and VCAM-1 (B) expression. TEC were stimulated for 18 hr with TNF-α (20 ng/mL) or IL-1 (20 ng/mL) in the presence or absence of DEX (4 and 40 μg/mL). ICAM-1 and VCAM-1 expression was assayed by cell ELISA. Data represent delta O.D. (mean ± SE) from two to nine different experiments (triplicate determinations).

Chemical Co (St Louis, MO). Azathioprine (AZA) was a gift from Burroughs Wellcome Co. (Research Triangle Park, NC), and cyclosporine A (CSA) form Sandoz Research Institute (East Hanover, NJ). The rat monoclonal antibody (mAb) M/K-2.7 (IgG_1) directed against murine VCAM-1 [11] was kindly provided by Dr Paul W. Kincade (Oklahoma City, OK). The hybridoma producing the rat IgG_{2a} mAb YN1/1.7.4 (anti-ICAM-1) [12] was from the American Type Culture Collection (ATCC, Rockville, MD). Recombinant murine TNF-α was provided by Genentech Inc. (South San Francisco, CA), and rmuIL-1 was donated by Hoffmann-LaRoche (Nutley, NJ).

SV40 transformed tubular epithelial cells (TEC) derived from C3H/FeJ mice (C1 cells) were grown in modified K1 media as described [1, 2, 13]. Cells were grown to confluence in collagen-coated 60-mm Petri dishes, and were stimulated with TNF-α (20 ng/mL) or IL-1 (20 ng/mL) for 18 hr. Inhibitors (DEX, 6-MP and CSA) were added 15 min prior to and during stimulation with TNF-α or IL-1.

Testing of immunosuppressants. To test the effectiveness of immunosuppressants we isolated splenocytes from normal C3H/HeJ mice and stimulated these cells with anti-CD3 mAb 145-2C11 (1:10 dilution of hybridoma supernatant) in 96-well plates (6×10^5 cells/well). Immunosuppressants were added in different concentrations at the beginning of the cultures. After 48 hr the cells were pulsed with [^{3}H]thymidine, and were harvested 18 hr later to measure proliferation.

Cell ELISA. ICAM-1 and VCAM-1 expression by TEC was analysed using a cell ELISA as described [2]. After cytokine stimulation the cells were trypsinized for 5 min at room temperature and washed once with phosphate-buffered saline containing 5% fetal calf serum. Cells were then incubated with primary antibody culture supernatant at 1:10 for 1 hr at 4°. Cells were then washed twice, and incubated with horseradish peroxidase-conjugated goat anti-rat Ig at 1:400 in phosphate-buffered saline/5% fetal calf serum for 1 hr at 4°. Cells were washed again, and the pellet was resuspended in

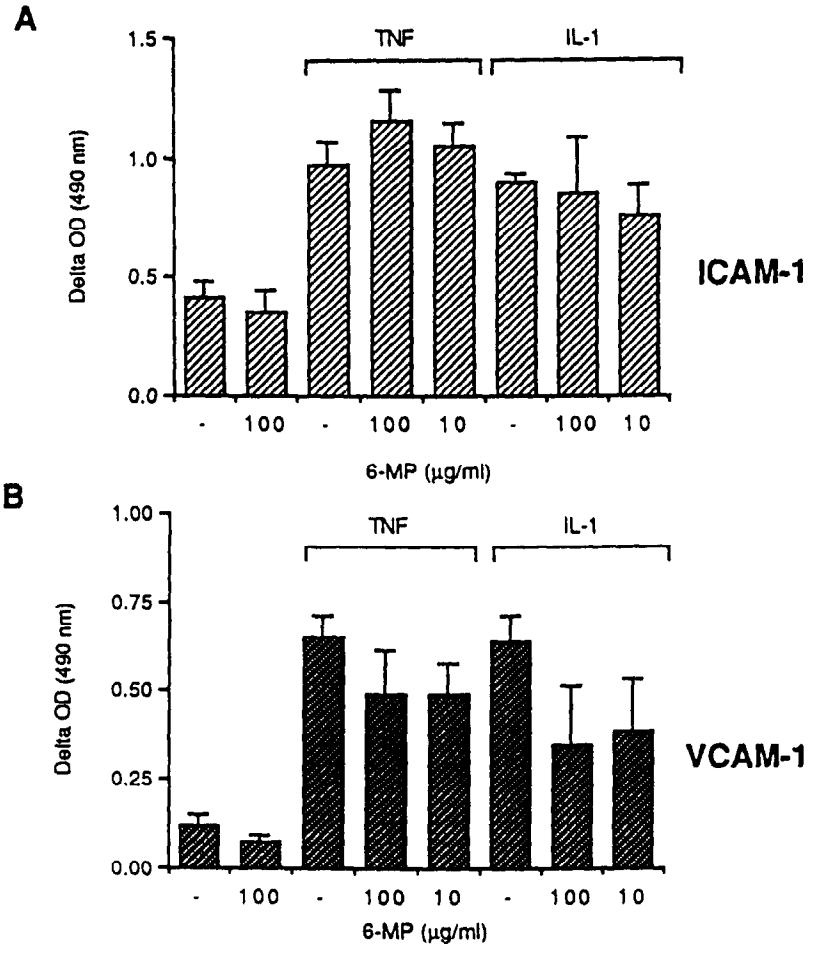

Fig. 2. Effect of 6-MP on TNF-α- and IL-1-stimulated ICAM-1 (A) and VCAM-1 (B) expression. TEC were stimulated with cytokines for 18 hr in the presence or absence of 6-MP (10 and 100 μg/mL). ICAM-1 and VCAM-1 expression was assayed by cell ELISA. Data represent delta O.D. (mean ± SE) from two to nine experiments (triplicate determinations).

0.1 M phosphate-citrate buffer (pH 5.0) containing 50 μg/mL of the substrate *o*-phenylenediamine dihydrochloride and 0.004% H_2O_2. After 8 min, 20% (v/v) of concentrated sulfuric acid was added to stop the reaction. Supernatants were than transferred to 96-well plates, and the O.D. was read at 490 nm on a Thermo Max microplate reader (Molecular Devices, Menlo Park, CA). Delta O.D. values were calculated by subtracting the O.D. of cells incubated without primary antibody. Means ± SE were then determined (N = 3).

RESULTS AND DISCUSSION

Proliferation of splenocytes in response to soluble anti-CD3 mAb was used to assess the effectiveness of the immunosuppressant drugs used in this study. Table 1 shows that soluble anti-CD3 mAb 145-2C11 induces a potent proliferation of unfractionated splenocytes. All the drugs tested were able to inhibit the proliferation of splenocytes dose dependently, demonstrating that they were effective immunosuppressants. Doses established in this assay were then used to study the effect on the expression of adhesion molecules by TEC.

Figures 1–3 demonstrate the effect of DEX (4 and 40 μg/mL), 6-MP (10 and 100 μg/mL) and CSA (0.1 and 1 μg/mL) on basal and cytokine-stimulated ICAM-1 and VCAM-1 expression by TEC. TNF-α and IL-1 (20 ng/mL) effectively upregulate ICAM-1 and VCAM-1 in these cells ($P < 0.01$). All three drugs had no significant effect on basal ICAM-1 and VCAM-1 expression ($P > 0.05$). Likewise, DEX, 6-MP and CSA did not significantly alter TNF-α- and IL-1-stimulated ICAM-1 and VCAM-1 expression in TEC ($P > 0.05$). AZA also did not significantly inhibit ICAM-1 and VCAM-1 (not shown).

In previous studies we have shown that ICAM-1 and VCAM-1 are upregulated in cultured renal TEC by the pro-inflammatory cytokines TNF-α, IL-1 and IFN-γ [1, 2, 13, 14]. In the present study we show that while the immunosuppressants DEX, 6-MP and CSA may effectively inhibit T cell responses and the production of adhesion molecule-inducing cytokines,

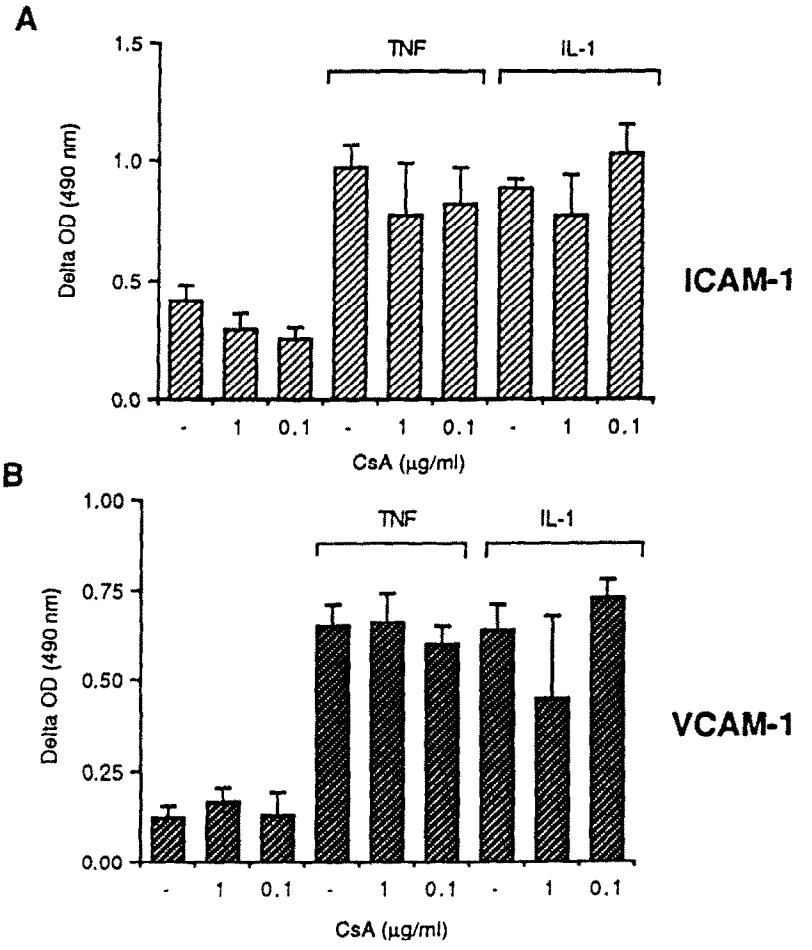

Fig. 3. Effect of CSA on TNF-α- and IL-1-stimulated ICAM-1 (A) and VCAM-1 (B) expression. TEC were stimulated for 18 hr with cytokines in the presence or absence of CSA (0.1 and 1 μg/mL). ICAM-1 and VCAM-1 expression was assayed by cell ELISA. Data represent delta O.D. (mean ± SE) from two to nine experiments (triplicate determinations).

they have no direct effect on the expression of adhesion molecules by renal tubular epithelium. These immunosuppressants may however inhibit cytokines *in vivo*, and can indirectly inhibit the adhesion molecules ICAM-1 and VCAM-1. Further studies will be necessary to examine the *in vivo* effect of DEX, 6-MP and CSA on renal tubular adhesion molecules.

The *in vitro* assay system described above may be useful to test immunosuppressants and other pharmacologic agents for their inhibitory effect on cell adhesion molecule expression. We have reported previously that TEC can be used to correlate the expression of ICAM-1 and VCAM-1 with the adherence of immune cells [2, 14]. Valuable information regarding adhesion molecule immunobiology may thus be gained in further experiments.

Acknowledgements—We thank Dr David G. Warnock for his support and encouragement. We also thank Genentech Inc. for supplying us with rmuTNF-α, and Hoffmann-LaRoche for rmuIL-1. We are grateful to Sandoz Pharmaceuticals and Wellcome Laboratories for providing CSA and AZA, respectively. We also thank Dr P. Kincade for providing the mAb M/K-2.7. This work was supported by NIH grant DK-39258, and the Nephrology Research and Training Center.

REFERENCES

1. Wuthrich RP, Jevnikar AM, Takei F, Glimcher LH and Kelley VE, Intercellular adhesion molecule-1 (ICAM-1) expression is upregulated in autoimmune murine lupus nephritis. *Am J Pathol* **136**: 441–450, 1990.
2. Wuthrich RP, Vascular cell adhesion molecule-1 (VCAM-1) expression in murine lupus nephritis. *Kidney Int* **42**: 903–914, 1992.
3. Springer TA, Adhesion receptors of the immune system. *Nature* **346**: 425–434, 1990.
4. Marlin SD and Springer TA, Purified intercellular adhesion molecule-1 (ICAM-1) is a ligand for lymphocyte function-associated antigen 1 (LFA-1). *Cell* **51**: 813–819, 1987.
5. Elices MJ, Osborn L, Takada Y, Crouse C, Luhowskyj S and Hemler ME, VCAM-1 on activated endothelium interacts with the leukocyte integrin VLA-4 at a site distinct from the VLA-4/fibronectin binding site. *Cell* **60**: 577–584, 1990.
6. Bishop GA and Hall BM, Expression of leukocyte and

lymphocyte adhesion molecules in the human kidney. *Kidney Int* **36**: 1078–1085, 1989.
7. Faull RJ and Russ GR, Tubular expression of intercellular adhesion molecule-1 during renal allograft rejection. *Transplantation* **48**: 226–230, 1989.
8. Lhotta K, Neumayer HP, Joannidis M, Geissler D and König P, Renal expression of intercellular adhesion molecule-1 in different forms of glomerulonephritis. *Clin Sci* **81**: 477–481, 1991.
9. Seron D, Cameron JS and Haskard DO, Expression of VCAM-1 in the normal and diseased kidney. *Nephrol Dial Transplant* **6**: 917–922, 1991.
10. Briscoe DM, Pober JS, Harmon BE and Cotran RS, Expression of vascular cell adhesion molecule-1 in human renal allografts. *J Am Soc Nephrol* **3**: 1180–1185, 1992.
11. Miyake K, Weissman IL, Greenberger JS and Kincade PW, Evidence for a role of the integrin VLA-4 in lymphohemopoiesis. *J Exp Med* **173**: 599–607, 1991.
12. Takei F, Inhibition of mixed lymphocyte response by a rat monoclonal antibody to a novel murine lymphocyte activation antigen (MALA-2). *J Immunol* **134**: 1403–1407, 1985.
13. Wuthrich RP, Glimcher LH, Yui MA, Jevnikar AM, Dumas SE and Kelley VE, MHC class II, antigen presentation and tumor necrosis factor in renal tubular epithelial cells. *Kidney Int* **37**: 783–792, 1990.
14. Jevnikar AM, Wuthrich RP, Takei F, Xu HW, Brennan DC, Glimcher LH and Rubin-Kelley VE, Differing regulation and function of ICAM-1 and class II antigens on renal tubular cells. *Kidney Int* **38**: 417–425, 1990.