Urol Res (1997) 25:231–238

ORIGINAL PAPER

K. Tanabe · S. C. Campbell · J. P. Alexander
F. Steinbach · M. G. Edinger · R. R. Tubbs
A. C. Novick · E. A. Klein

Molecular regulation of intercellular adhesion molecule 1 (ICAM-1) expression in renal cell carcinoma

Received: 14 March 1996 / Accepted: 27 October 1996

Abstract Intercellular adhesion molecule-1 (ICAM-1) mediates two important functional aspects of tumor biology, namely enhancement of tumor metastasis and mediation of host defense mechanisms such as lymphocyte-mediated tumor cytotoxicity. Since ICAM-1 is expressed by most renal cell carcinomas (RCC), the regulation of ICAM-1 expression is important in understanding the biological behavior of RCC. We report an investigation on ICAM-1 expression and molecular regulation by cytokines and protein kinase C activator on RCC cell lines. Of the various cytokines, tumor necrosis factor α (TNFα), interferon-γ (IFNγ), and phorbol myristate acetate (PMA) strongly upregulated ICAM-1 protein expression on RCC. The kinetics of ICAM-1 message induction was studied by Northern analysis of total RNA extracted from RCC and normal kidney proximal tubular (NKPT) cells. Time course studies showed that ICAM-1 mRNA was upregulated by INFγ, TNFα, and PMA, plateaued after 2 h, and remained increased for up to 24 h. Although ICAM-1 mRNA in NKPT cells was upregulated by these cytokines, their messages returned to basal levels after 24 h. ICAM-1 mRNA stability assays showed that both unstimulated and stimulated RCC cells had very stable ICAM-1 mRNA up to 24 h. In order to investigate whether increased gene transcription contributes to ICAM-1 upregulation, RCC cells were treated with TNFα, IFNγ, or PMA with or without simultaneous addition of actinomycin D. ICAM-1 message induction-blocking studies suggested that primary upregulation of ICAM-1 mRNA may be caused by transcriptional upregulation. These results suggest that long-lasting ICAM-1 message upregulation in response to cytokines or PMA may be due to transcriptional upregulation in the early phase and stabilization of ICAM-1 message in the later phase (after 4 h). These observations suggest that RCC may lack the normal downregulatory mechanisms which control ICAM-1 expression and may explain the high frequency of ICAM-1 expression observed on primary human RCC.

Key words ICAM-1 · Molecular regulation · Renal cell carcinoma · Cytokines · Protein kinase C activator

K. Tanabe (✉)
Department of Urology, Kidney Center,
Tokyo Women's Medical College,
8-1 Kawada-cho, Shinjuku-ku, Tokyo 162 Japan

S. C. Campbell · J. P. Alexander · F. Steinbach
A. C. Novick · E. A. Klein
Department of Urology, The Cleveland Clinic Foundation,
9500 Euclid Avenue, Cleveland, OH 44195, USA

M. G. Edinger · R. R. Tubbs
Department of Pathology, The Cleveland Clinic Foundation,
9500 Euclid Avenue, Cleveland,
OH 44195, USA

Introduction

Intercellular adhesion molecule-1 (ICAM-1, CD54), one of the ligands of lymphocyte function-associated antigen-1 (LFA-1), is a 90- to 114-kDa sialoglycoprotein member of the immunoglobulin gene superfamily which is constitutively expressed on hematopoietic cells, vascular endothelium, fibroblasts, and some epithelial cells [12, 27, 38]. ICAM-1 expression has also been demonstrated on malignant cells such as melanomas [1, 22, 32], lymphomas [4, 17, 39], neuroblastoma [30], and other carcinomas [30, 42, 44].

During analysis of melanoma, Johnson et al. [22] determined that one of the melanoma-associated antigens, P3.58, an 89-kDa cell surface glycoprotein which is expressed predominantly in metastatic lesions, is identical to ICAM-1 [22]. They showed that ICAM-1 was detectable on advanced human melanoma, but not on benign melanocytes or early melanoma, and the frequency of ICAM-1 expression correlated with melanoma thickness, and suggested that ICAM-1 expression may contribute to the metastatic or invasive capacity of melanoma cells [22]. Natali et al. [31] confirmed that expression of ICAM-1 in primary melanoma correlated

with the thickness of the lesions and showed that the frequency of expression of ICAM-1 was markedly higher in metastatic lesions than in primary melanomas [31]. They concluded that ICAM-1 might be a useful marker to investigate the molecular mechanisms of the biological behavior of melanoma [31].

In addition, the importance of ICAM-1/LFA-1 interaction in lymphocyte function such as cytotoxicity and T-cell proliferation has been demonstrated in a variety of studies [11, 16, 19, 24, 35, 37], including ICAM-1/LFA-1-mediated T-cell killing [6, 8, 14, 20, 28, 30, 43]. For example, Jackson et al. [20] reported that some transitional cell carcinoma (TCC) cell lines express ICAM-1, and that interferon-γ (IFNγ) and tumor necrosis factor α (TNFα) can augment ICAM-1 expression [20]. After IFNγ treatment, ICAM-1 expression on TCC cell lines and lymphocyte-activated killer (LAK) cell-mediated killing clearly correlated in a dose-dependent manner and this LAK cell-mediated cytotoxicity could be blocked by anti-ICAM-1 monoclonal antibody [20]. These findings suggest that T cells infiltrating into the bladder wall after bacillus Calmette-Guérin (BCG) treatment released cytokines and upregulated ICAM-1 expression on tumor cells, and as a result lymphocyte-mediated cytotoxicity could be augmented.

Recently, we reported that more than 90% of renal cell carcinomas (RCC) expressed high levels of ICAM-1 by immunohistological and flow cytometric analysis, while corresponding normal kidney proximal tubular (NKPT) epithelium does not [41]. Since RCC is relatively responsive to immunotherapy with cytokines [7], LAK cells [33], and tumor-infiltrating lymphocyte (TIL) cells [2, 23], we also examined the functional role of ICAM-1 on RCC and found that ICAM-1 was important in TIL/RCC interactions including TIL-mediated cytotoxicity and TIL proliferation [41]. Because ICAM-1 is expressed by most RCC and because it is potentially important in several functional aspects of tumor biology (metastasis and host defense mechanisms), we investigated the molecular regulation of ICAM-1 by RCC and normal kidney. We described the molecular regulatory mechanisms in RCC underlying the inducible expression of ICAM-1 by various cytokines and protein kinase C activators, and conclude that RCC upregulates ICAM-1 expression in response to inflammatory cytokines but exhibits a defect in the downregulatory mechanisms present in normal proximal tubular cells.

Materials and methods

Human RCC cell lines

The RCC cell lines RC-1 and RC-2 [18] were maintained in complete RPMI media [10% fetal calf serum (FCS), 200 mM L-glutamine, 25 mM hydroxyethylpiperazine ethanesulfonic acid (HEPES), 100 mM sodium pyruvate, 10 mM non-essential amino acids and 5×10^{-5} M 2-mercaptoethanol]. Cells were trypsinized weekly and grown to subconfluency for experiments.

Short-term culture of NKPT cells

The kidney specimens were perfused with 500 ml Hank's balanced salt solution (HBSS, Whittaker Bioproducts, Walkersville, MD) or saline to remove extraneous blood and processed as described [13]. The normal kidney tissue was minced and digested with collagenase type II solution [1 mg/ml (Sigma, St. Louis, MO) in Dulbecco's modified Eagle's medium (DMEM, Whittaker Bioproducts, Walkersville, MD)] containing trypsin inhibitor solution (3.5 mg/ml in DMEM, 385 u/mg, Sigma) for 1.5 h at 37°C. NKPT cells were placed in complete RPMI (10% FCS, 2 mM L-glutamine, 10 mM HEPES, 1 mM sodium pyruvate, 0.1 mM non-essential amino acids and 5×10^{-5} M 2-mercaptoethanol) and incubated at 37°C in 5% CO_2 and 95% humidity. NKPT cells were grown to subconfluency and harvested for mRNA extraction. At the same time, the cells were immunostained for cytokeratin and Uro-5 (Signet, MA) to ensure the cells were of epithelial origin and not fibroblastic.

Reagents

Monoclonal anti-ICAM-1 antibody was purchased from Becton-Dickinson (Mountain View, CA). Cytokines used in this study are as follows: human recombinant (r) IL-1β (R&D, Minneapolis, MN), rIL-2 (Hoffmann-La Roche, Nutley, NJ), rIL-3 (Sandoz, Austria), rIL-4 (Sterling, Malvern, PA), rIL-5 (R&D, Minneapolis, MN), rIL-6 (Sandoz, Austria), r-interferon-α (IFNα) (Schering, Kenilworth, NJ), rIFNγ (Biogen, Cambridge, MA), and rTNFα (Cetus Corporation, Emeryville, CA). Phorbol myristate acetate (PMA, Sigma) was reconstituted with dimethylsulfoxide (DMSO)/RPMI and stored at −70°C until usage.

Two-color immunocytometry

Immunocytometric analysis of cultured RCC was performed using two-color immunofluorescence [26]. Fluorescein isothiocyanate (FITC) and phycoerythrin-conjugated monoclonal antibody (mAb) were employed to phenotypically identify and quantify tumor cell subsets. Isotypic controls for each particular subclass of immunoglobulin were utilized. Analyses on the FACScan (Becton-Dickinson, San Jose, CA) were performed utilizing an argon ion laser (Cyonics, San Jose, CA) with 15 mW of 488 nm excitation. Optics in the fluorescence path included a focusing objective, beam splitters, dichroic mirrors, a 530-nm bandpass filter, a 585-nm bandpass filter, and a 650-nm-long pass/cut-on filter. Analysis was done using multicontour, multiparameter software [Paint-a-Gate and LYSIS II (Becton-Dickinson)]. Results were reported as a tumor cell percentage corrected for nonspecific binding of isotypic controls as determined by measurement of autofluorescent background.

Plasmid

pCDM8 containing ICAM-1 cDNA was kindly provided by Dr. T. Springer (Harvard Medical School, MA) and digested with *Xba*I to generate a 1.8-kb ICAM-1 cDNA.

RNA isolation and Northern analysis

Total RNA was extracted from unstimulated RCC cells and short-term cultured NKPT cells and following stimulation with cytokines or PMA using the guanidine isothiocyanate cesium chloride method [45]. Briefly, the medium was removed, and cells were washed with HBSS (Gibco-BRL, Gaithersburg, MD) and lysed with 5.2 ml guanidine solution (4.2 M guanidine isothiocyanate, 0.17 M *N*-lauroylsarcosine, 25 mM sodium citrate, 90 mM 2-mercaptoethanol). The lysate was drawn repeatedly through a 5-ml pipette and layered over a 2.0-ml, 5.7 M cesium chloride (CsCl) cushion. RNA was purified by centrifugation in a Sorvall SW41 Ti rotor (Beckman) for 14–20 h at 30 000 rpm. The RNA pellet was

resuspended in diethylpyrocarbonate (DEPC)-treated water and subjected to ethanol precipitation. RNA was quantified spectrophotometrically by determining absorbance at 260 nm. RNA samples (20–30 μg/lane) were subjected to electrophoresis in denaturing 1% formaldehyde-agarose gels and transferred to nylon membranes (MSI, MA) overnight in 20 × sodium saline citrate (SSC) (3 M NaCl, 0.3 M sodium citrate, pH 7.0). RNA was fixed to the membrane by ultraviolet cross-linking (Stratagene, La Jolla, CA). Blots were prehybridized for at least 6 h at 42°C in 50% formamide, 6 × SSC, 0.1% sodium dodecyl sulfate (SDS), 5 × Denhardt's solution (0.1% bovine serum albumin, Ficoll, and polyvinyl pyrolidine), and 250 mg denatured, sonicated herring sperm DNA. Blots were hybridized at 42°C for 16–24 h using random-primed (Boehringer-Manheim), ^{32}P-labeled ICAM-1 cDNA at a final concentration of 1.0×10^6 dpm/ml. Blots were washed for 20 min at room temperature in 1 × SSC, 0.2% SDS, followed by a second 20-min wash at 65°C in 1 × SSC, 0.2% SDS and a final wash with 0.1 × SSC, 0.2% SDS at 65°C. The blots were exposed to Kodak XAR-5 film at −70°C using an intensifying screen for 24–48 h. In order to normalize mRNA content, blots were stripped and reprobed with a human β-actin oligoprobe (40 bp, synthesized by the Department of Molecular Biology, Cleveland Clinic Foundation).

Time course of ICAM-1 mRNA induction

In order to directly investigate the kinetics of ICAM-1 mRNA induction by IFNγ (500 u/ml), TNFα (500 u/ml), and PMA (10 ng/ml), total RNA was extracted at various time points from RCC cells or NKPT cells incubated with IFNγ, TNFα, or PMA, and subjected to Northern analysis using a 1.8-kb ICAM-1 cDNA probe. ICAM-1 mRNA was quantified by densitometry of autoradiograms and normalized to β-actin mRNA content.

ICAM-1 mRNA stability assay

In order to investigate the ICAM-1 mRNA stability, RCC cells were treated with TNFα (500 u/ml for 4 h), IFNγ (500 u/ml for 4 h), or PMA (10 ng/ml for 4 h) and subsequently actinomycin D (Sigma, 10 μg/ml in DMSO) was added to inhibit all new transcription. The decay of ICAM-1 mRNA was examined up to 24 h.

ICAM-1 mRNA induction-blocking assay

In order to investigate whether transcriptional upregulation contributes to the ICAM-1 mRNA upregulation, RCC cells were treated with TNFα (500 u/ml), IFNγ (500 u/ml), or PMA (10 ng/ml) with or without simultaneous addition of actinomycin D (10 μg/ml). Total RNA was extracted 0, 2, and 4 h later and Northern analysis was performed.

Results

Various cell activators upregulate ICAM-1 expression on RCC

RC-1 and RC-2 cells constitutively expressed ICAM-1 including 90% of RC-1 and 35% of RC-2 (control, Fig. 1a, b). While the percentage on RC-1 did not increase with cytokine treatment (Fig. 1a), the intensity or density of expression did increase following stimulation with TNFα, IFNγ, PMA, or IFNα (Fig. 1c). Upon stimulation with IFNα, IFNγ, TNFα, or PMA, the percentage of ICAM-1 expression on RC2 increased by more than double (Fig. 1b). The intensity of expression on RC-2 also increased with TNFα or IFNγ stimulation (Fig. 1d). Treatment with rIL-1β, rIL-2, rIL-3, rIL-4, rIL-5, and rIL-6 did not upregulate ICAM-1 protein expression in either RC-1 or RC-2. Of all the activators that induce ICAM-1 expression, IFNα was the least effective while the largest inductions were caused by TNFα, IFNγ, and PMA.

Time course of ICAM-1 mRNA induction

In order to investigate the kinetics of ICAM-1 message induction by TNFα, IFNγ, and PMA, total RNA was extracted from RCC cells and NKPT cells, and Northern analysis was performed. ICAM-1 mRNA was detected in RC-1 cells and NKPT cells under unstimulated conditions (Fig. 2A, lane 0, Fig. 3, lane 0). After 2 h incubation with either TNFα or IFNγ, ICAM-1 mRNA was upregulated in RCC cells, reached a plateau level at 2 h and remained high for up to 24 h. Both RC-1 and RC-2 cells showed the same pattern of ICAM-1 mRNA kinetics. The representative time course of Northern blot of RC-1 cells is shown in Fig. 2A, B. On the other hand, ICAM-1 mRNA in NKPT cells was upregulated after cytokine treatment, and returned to almost basal levels within 24 h. In three individual experiments, ICAM-1 mRNA expression in TNFα-treated NKPT cells reached a peak level after 2 h and returned to basal levels after 24 h while ICAM-1 mRNA induced by IFNγ or PMA treatment reached a peak level at 4 h and returned to almost basal levels in two of three experiments. The representative time course-Northern blot of NKPT cells is shown in Fig. 3.

ICAM-1 mRNA stability assay

Since ICAM-1 mRNA was upregulated for a long time (up to 24 h, maximum time point), message stability was measured to determine the mechanism of increased ICAM-1 mRNA expression. RC-1 cells in which ICAM-1 mRNA was detected constitutively were treated with IFNγ, TNFα, or PMA, and actinomycin D (10 μg/ml) was added at 4 h to inhibit new transcription. After addition of actinomycin D, the decay of ICAM-1 mRNA was examined up to 24 h (Fig. 4A, B). ICAM-1 mRNA from unstimulated RC-1 cells did not decay up to 24 h after actinomycin D treatment, demonstrating that constitutively expressed ICAM-1 mRNA was very stable. ICAM-1 mRNA from IFNγ, TNFα, or PMA-stimulated RC-1 cells also showed a very stable message (Fig. 4A, B).

ICAM-1 mRNA induction-blocking assay

In order to investigate whether increased gene transcription contributes to the ICAM-1 mRNA upregula-

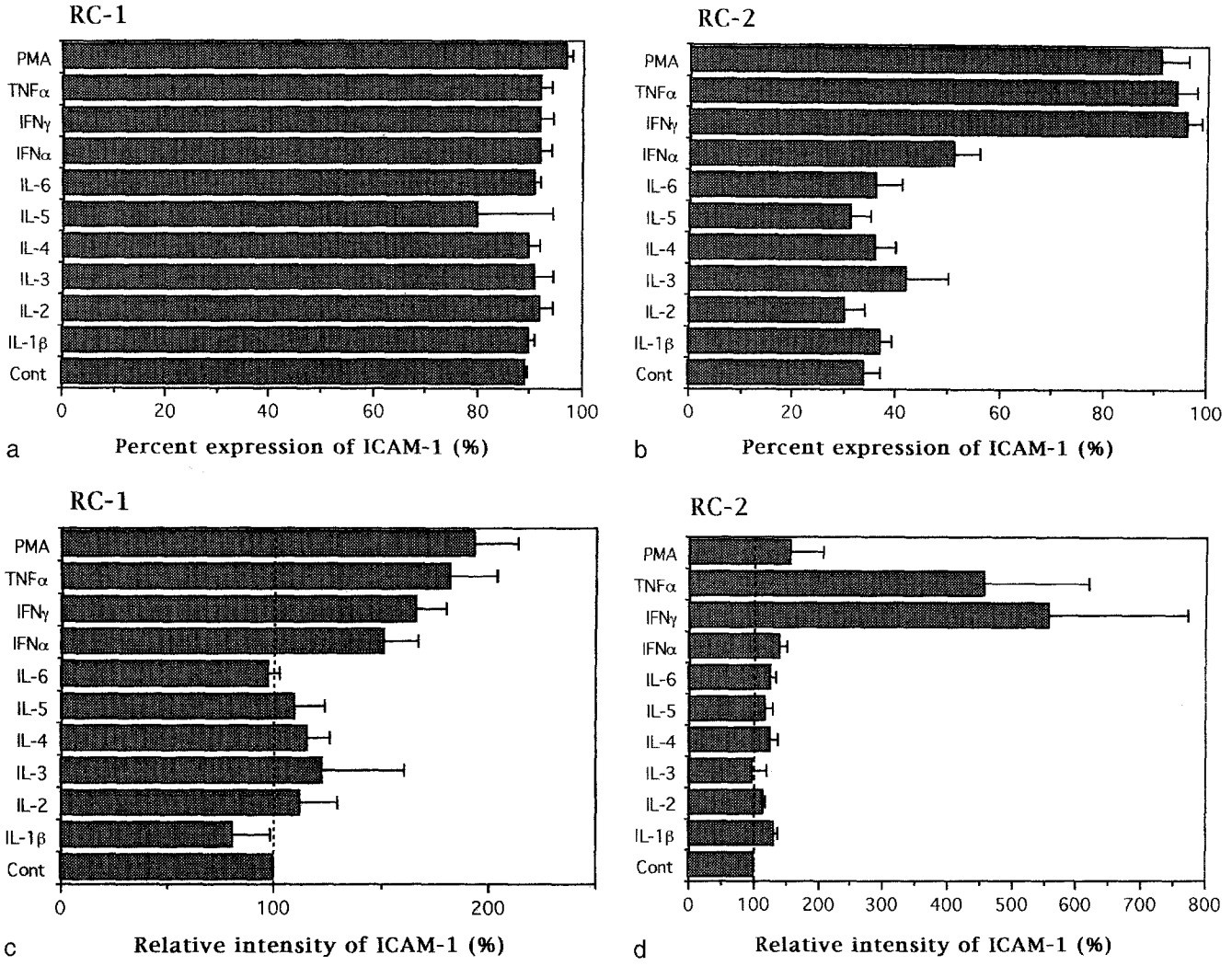
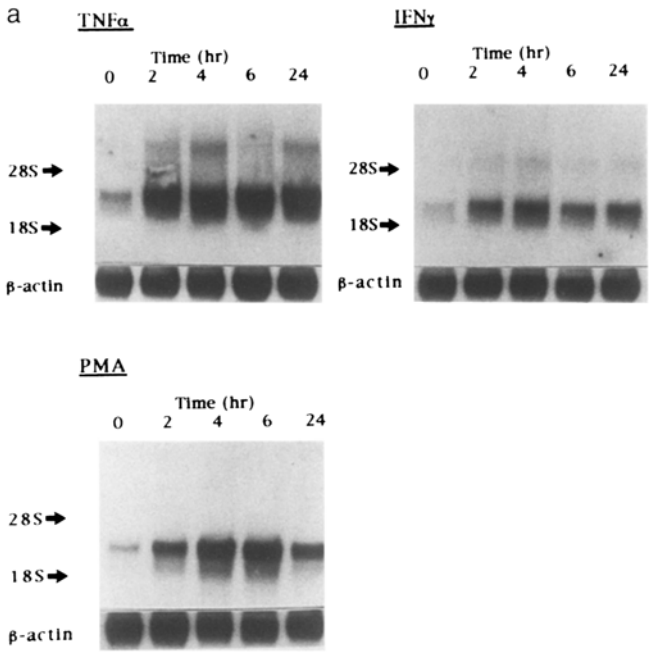


Fig. 1 Flow cytometric analysis of ICAM-1 expression on RCC lines. RC-1 and RC-2 cells were treated with rIL-1β (500 u/ml), rIL-2 (500 u/ml), rIL-3 (500 u/ml), rIL-4 (500 u/ml), rIL-5 (500 u/ml), rIL-6 (500 u/ml), rIFNγ (500 u/ml), IFNα (500 u/ml), TNFα (500 u/ml), and PMA (10 ng/ml). After 24 h, the cells were trypsinized, and ICAM-1 protein expression was analyzed by flow cytometry as described in "Materials and methods." Percentage expression of ICAM-1 was expressed as the percentage of ICAM-1-positive cells. Percentage intensity of ICAM-1 was expressed as the percentage of ICAM-1 expression on unstimulated RC-1 or RC-2 cells. All values were the average of four individual experiments

tion, RC-1 cells were treated with TNFα, IFNγ, or PMA with or without simultaneous addition of actinomycin D (10 μg/ml). Total RNA was extracted at 0, 2, and 4 h and Northern analysis was carried out. After cytokine or PMA treatment without actinomycin D, the level of ICAM-1 mRNA expression increased significantly. Under simultaneous actinomycin D treatment, no upregulation was observed (Fig. 5). These findings suggest that increased transcription of ICAM-1 mRNA might be an important mechanism in the upregulation of ICAM-1 mRNA during the early phase (2–3 h) of induction.

Discussion

Observations in several tumor systems suggest that ICAM-1 subserves two functions in tumor biology, including a role in enhancement of metastasis and in mediating host defense mechanisms such as lymphocyte-mediated tumor cytotoxicity [2, 20, 22, 23, 31, 33, 41, 43]. We have previously demonstrated that most primary human RCC express high levels of ICAM-1 while corresponding normal kidney proximal tubular cells do not, and that ICAM-1 expression by RCC mediates important biological and immunological functions [41]. The present study examines the molecular regulation of ICAM-1 expression by RCC and NKPT cells. Treatment of RCC with various cytokines or protein kinase C activator PMA revealed that IFNα, IFNγ, TNFα, and PMA could upregulate ICAM-1 protein expression on RCC cell lines (Fig. 1). Conversely, IL-1β, IL-2, IL-3, IL-4, IL-5, and IL-6 did not upregulate ICAM-1 expression. These observations parallel findings of other reports that demonstrate that IFNγ, TNFα, PMA, and lipopolysaccharide (LPS) can augment ICAM-1 ex-



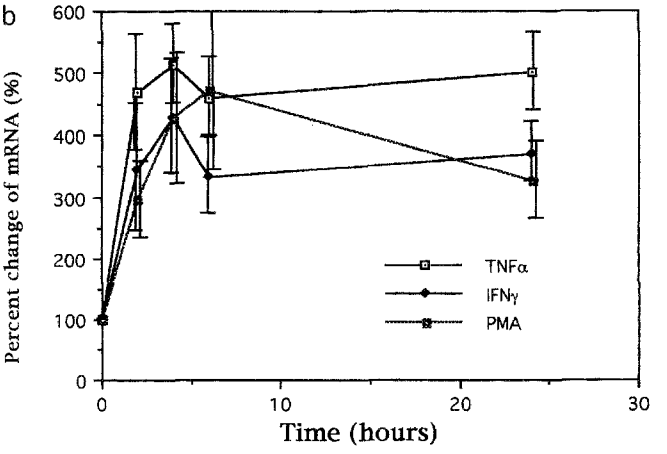


Fig. 2a, b Time course of ICAM-1 mRNA induction of RC-1 by IFNγ, TNFα, and PMA. RC-1 cells were treated with IFNγ (500 u/ml), INFα (500 u/ml), or PMA (10 ng/ml) for the time indicated. **a** Total RNA was extracted from each sample, and 20 μg RNA per lane was loaded for Northern analysis as described in "Materials and methods." **b** ICAM-1 mRNA was quantified by densitometry of autoradiograms and normalized to β-actin mRNA content

pression on various kinds of cells including human umbilical vein endothelial cell (HUVEC) [15, 45], fibroblasts [4, 34], and many kinds of carcinomas [6, 9, 14, 20, 25, 30]. Contrary to reports that IL-1β can upregulate ICAM-1 expression on fibroblasts [4, 34], HUVEC [15, 45], and some epithelial tumors [6, 21, 25, 34, 47], we observed no effect of this cytokine on ICAM-1 expression in our RCC cell lines. A recent report suggests that RCC cells may produce an IL-1 receptor antagonist as seen in keratinocytes and the lack of response to IL-1β may be explained by the occupation of the IL-1 receptor by an autologously produced antagonist [3].

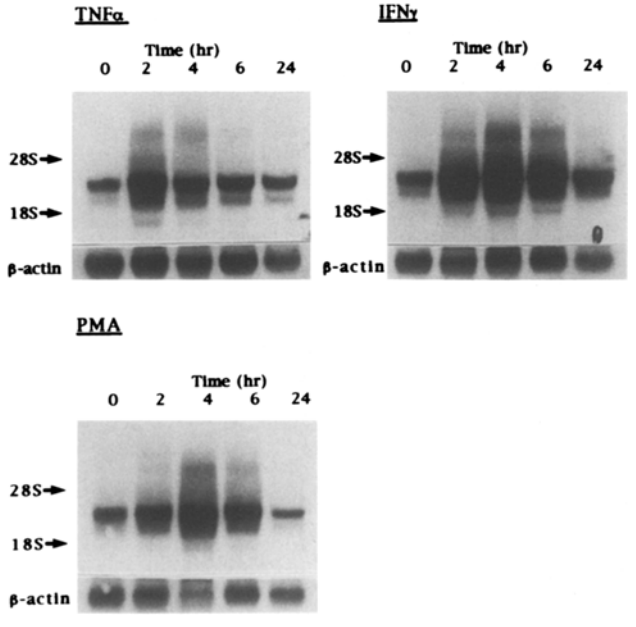


Fig. 3 Time course of ICAM-1 mRNA induction of NKPT cells by IFNγ, TNFα, and PMA. NKPT cells were treated with IFNγ (500 u/ml), TNFα (500 u/ml), or PMA (10 ng/ml) for the time indicated. Total RNA was extracted from each sample, and 20 μg of RNA per lane was loaded for Northern analysis as described in "Materials and methods"

Following these observations, we performed time course studies in order to directly investigate the kinetics of ICAM-1 message induction by cytokine stimulators. We observed a rapid increase in ICAM-1 mRNA expression after stimulation by cytokines and PMA, with continued high levels of expression 24 h after stimulation (Fig. 2). In contrast, ICAM-1 mRNA in NKPT cells was upregulated after cytokine treatment, peaked between 2 and 4 h, and returned to basal levels within 24 h (Fig. 3). The kinetics of ICAM-1 mRNA induction in NKPT appears to parallel that reported in other model systems. Wertheimer et al. [45] reported that ICAM-1 mRNA in HUVEC reached peak levels by 2–4 h after PMA or TNFα stimulation, and normalized by 24 h. Gerritsen et al. [15] reported that after combined treatment with TNFα and IFNγ, ICAM-1 mRNA was observed as early as 2 h, attained maximal levels between 4 and 8 h, and declined by 24 h in both HUVEC and human synovial microvascular endothelial cells [15]. These observations demonstrate that RCCs are more sensitive to cytokine-induced ICAM-1 mRNA transcription and may be deficient in the normal downregulatory mechanisms which control ICAM-1 message expression in normal cells. This regulatory dysfunction may in part account for the high expression of ICAM-1 seen in RCC compared with NKPT [41]. Other tumors appear to regulate ICAM-1 transcription normally. Kuppner et al. [25] reported peak ICAM-1 expression by glioblastoma in response to TNFα and IL-

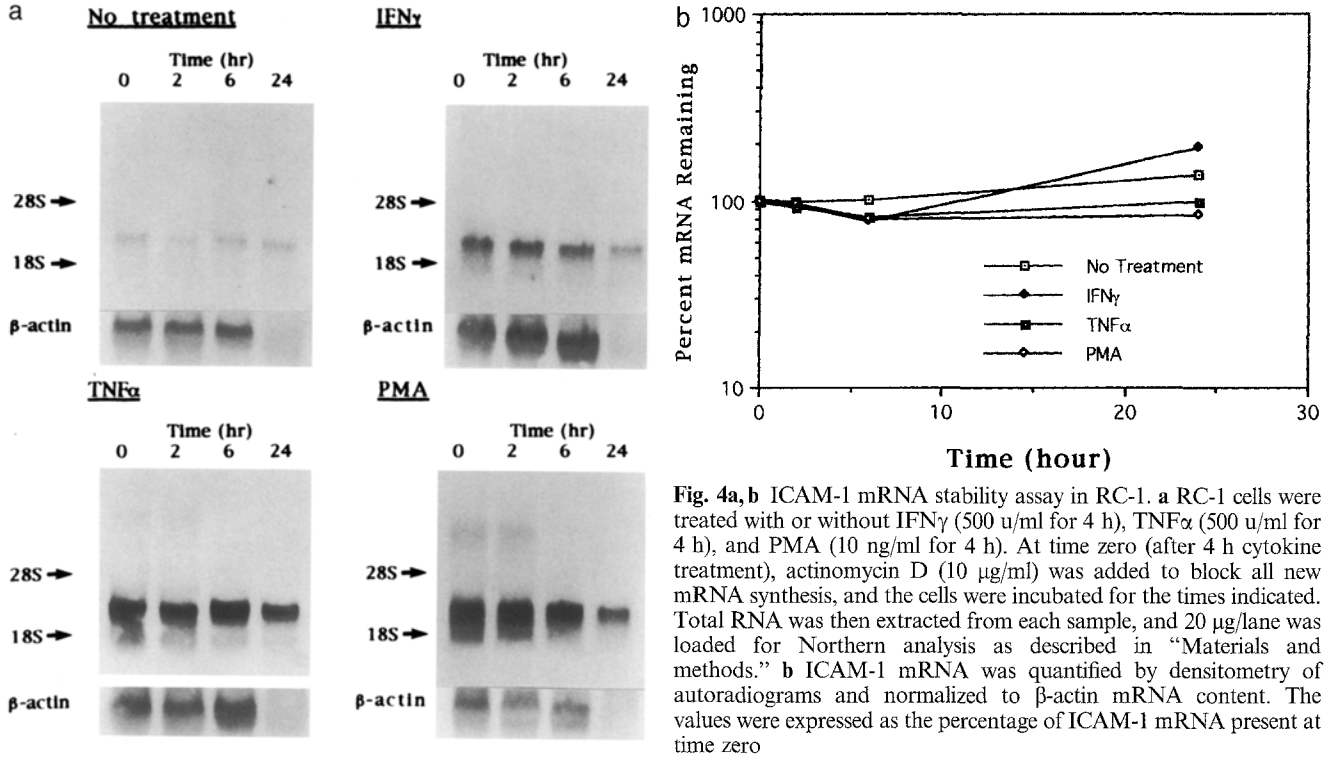


Fig. 4a, b ICAM-1 mRNA stability assay in RC-1. **a** RC-1 cells were treated with or without IFNγ (500 u/ml for 4 h), TNFα (500 u/ml for 4 h), and PMA (10 ng/ml for 4 h). At time zero (after 4 h cytokine treatment), actinomycin D (10 μg/ml) was added to block all new mRNA synthesis, and the cells were incubated for the times indicated. Total RNA was then extracted from each sample, and 20 μg/lane was loaded for Northern analysis as described in "Materials and methods." **b** ICAM-1 mRNA was quantified by densitometry of autoradiograms and normalized to β-actin mRNA content. The values were expressed as the percentage of ICAM-1 mRNA present at time zero

1β at 4 h after cytokine treatment, with return to baseline at 24 h [25].

Next we examined the details of molecular regulation of ICAM-1 mRNA in RCC. No upregulation of ICAM-1 mRNA was noted when RCC cells were stimulated by cytokines or PMA simultaneously with actinomycin D (Fig. 5). This finding suggests that transcriptional upregulation might be crucial in the early phase of ICAM-1 mRNA induction (within 2–3 h after stimulation). These findings seem to be consistent with other observations that transcriptional upregulation is important for cytokine-stimulated induction of ICAM-1 message [45, 47]. In the stability assays (Fig. 4) we found that ICAM-1 message was stable for a relatively long time period despite the fact that new transcription was blocked by actinomycin D at 4 h after stimulation. These findings suggest that transcriptional upregulation may not be essential after 4 h of cytokine stimulation. Furthermore, ICAM-1 mRNA was found to be extremely stable when compared with other cells such as HUVEC [15, 45]. Wertheimer et al. [45] reported that ICAM-1 mRNA in HUVEC was stable after treatment with PMA, but not with TNFα. Since the gene exhibits sufficient basal transcriptional activity to support low-level ICAM-1 protein biosynthesis in unstimulated HUVEC [29], and constitutively expressed message is undetectable by Northern analysis, they speculated that ICAM-1 mRNA is probably unstable in HUVEC [45]. In contrast, we observed constitutive ICAM-1 mRNA expression in RCC even under nonstimulatory conditions, and that ICAM-1 mRNA was stable up to 24 h after the blockage of new transcription by actinomycin D (Fig. 4). These results clearly show that ICAM-1 mRNA in RCC is stable even in unstimulated cells, and that after cytokine or PMA stimulation upregulated mRNA was accumulated by its stability. This regulation in RCC seems to be different from that observed in HUVEC, as Wertheimer et al. [45] concluded that PMA itself caused the stabilization of ICAM-1 mRNA [45].

Although ICAM-1 mRNA in RCC is much more stable when compared with normal tissue such as HUVEC [15, 45] and NKPT cells, we do not have enough data to explain the mechanism by which this occurs. Several studies have demonstrated that the presence of AUUUA sequences in the 3′-untranslated region (3′UTR) of a variety of eukaryotic mRNAs correlates with rapid mRNA degradation [5, 10]. For example, deletion of AU-rich sequences from the c-*fos* 3′UTR confers stability upon transcripts produced from transfected constructs [5, 46]. Furthermore, introduction of a 51-nucleotide AU-rich sequence from the granulocyte-macrophage colony-stimulating factor (GM-CSF) 3′UTR of the rabbit β-globin gene confers instability upon the otherwise stable β-globin mRNA [36]. Bohjanen et al. [40] reported that cytoplasmic factor (AU-B), which binds to AU-rich sequences in 3′UTRs, prevents this rapid mRNA degradation [5]. ICAM-1 has two AUUUA motifs in the 3′UTR [40], and during the process of carcinogenesis malignant cells may obtain de novo mRNA-binding proteins which bind to AU-rich sequences in the 3′UTR and as a result prevent degradation of ICAM-1 mRNA. Although this speculation seems to be the most likely explanation for the long-lasting stabilization of ICAM-1 message in RCC, further investigation is necessary to fully understand this phenomenon.

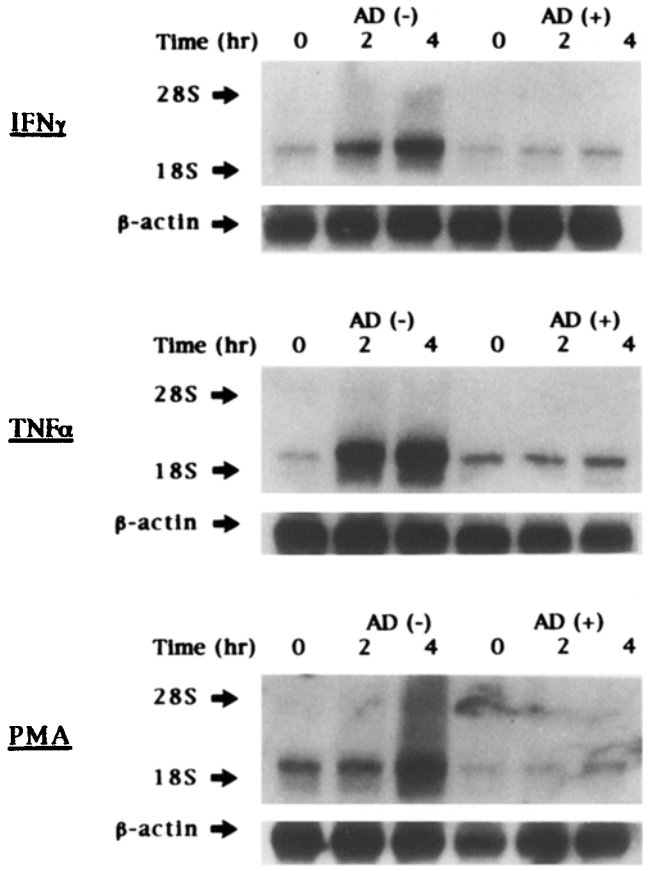


Fig. 5 ICAM-1 mRNA induction blocking assay. RC-1 cells were treated with TNFα (500 u/ml), IFNγ (500 u/ml), or PMA (10 ng/ml) at time zero, and also actinomycin D (10 μg/ml) was added at time zero. After 2 h and 4 h incubation, total RNA was extracted from each sample. For control, after TNFα, IFNγ, or PMA treatment, no actinomycin D was added and total RNA was extracted at time 0, 2, and 4 h. A quantity of 20 μg/lane of total RNA was loaded for Northern analysis as described in "Materials and Methods." *AD*(+) actinomycin D was added at time zero, *AD* (−) no actinomycin D was added

In summary, transcriptional upregulation of ICAM-1 mRNA might play an important role in the early phase of induction (first 2–3 h), and stabilization of ICAM-1 mRNA contributes to its prolonged upregulation in the later phase (after 4 h) in RCC cell lines. Contrary to these findings, ICAM-1 mRNA expression in response to cytokines peaked at 4 h and returned to baseline levels at 24 h in NKPT cells. This observation suggests that renal cell carcinomas exhibit a deficit in the normal downregulatory mechanisms which regulate ICAM-1 message levels. Our kinetic studies suggest that both transcriptional upregulation by inflammatory cytokines found in the tumor bed and prolonged message stability account for this observation. Further studies will be directed at elucidating the mechanism of message stability in RCC and on further biologic and functional immunologic effects due to ICAM-1 overexpression.

Acknowledgement We thank Teresa Bendele and Matt Owen for assistance with two-color cytometry staining and analysis and Dr. Yoshihiro Ohomori for reviewing the manuscript.

References

1. Becker JC, Dummer R, Hartmann AA, Burg G, Schmidt RE (1991) Shedding of ICAM-1 from human melanoma cell lines induced by IFN-γ and tumor necrosis factor-α. J Immunol 147:4398
2. Belldegrum A, Muul LM, Rosenberg SA (1998) Interleukin 2 expanded tumor-infiltrating lymphocytes in human renal cell cancer: Isolation, characterization, and antitumor activity. Cancer Res 48:206
3. Bigler CF, Norris D, Weston WL, Arend WP (1992) Interleukin-1 receptor antagonist production by human keratinocytes. J Invest Dermatol 98:38
4. Billaud M, Calender A, Seigneurin J-M, Lenoir GM (1987) LFA-1, LFA-3, and ICAM-1 expression in Burkitt's lymphoma. Lancet ii:1327
5. Bohjanen PR, Betryniak B, June CH, Thompson CB, Lindsten T (1991) An inducible cytoplasmic factor (AU-B) binds selectively to AUUUA multimers in 3′ untranslated region of lymphokine mRNA. Mol Cell Biol 11:3288
6. Boyd AW, Wawryk SO, Burns GF, Fecondo JV (1988) Intercellular adhesion molecule 1 (ICAM-1) has a central role in cell-cell contact-mediated immune mechanisms. Proc Natl Acad Sci USA 85:3095
7. Budd GT, Osgood B, Barna B, Boyett JM, Finke J, Medendorp SV, Murthy S, Novak C, Sergi J, Tubbs R, Bukowski RM (1989) Phase I clinical trial of interleukin 2 and α-interferon: Toxicity and immunologic effects. Cancer Res 49:6432
8. Busson P, Zhang Q, Guillon J-M, Gregory CD, Young LS, Clausse B, Lipinski M, Rickinson AB, Tursz T (1992) Elevated expression of ICAM1 (CD54) and minimal expression of LFA3 (CD58) in Epstein-Barr-virus-positive nasopharyngeal carcinoma cells. Int J Cancer 50:863
9. Campbell SC, Tanabe K, Alexnder JP, Edinger M, Tubbs RR, Klein EA (1994) Intercellular adhesion molecule-1 expression by bladder cancer cells: functional effects. J Urol 151:1385
10. Cleveland DW, Yen TJ (1989) Multiple determinants of eukaryotic mRNA stability. New Biol 1:121
11. Desroches CV, Rigal D, Andreoni, C (1991) Regulation and functional involvement of distinct determinants of leukocyte function-associated antigen 1 (LFA-1) in T-cell activation in vitro. Scand J Immunol 33:277
12. Dustin ML, Rothlein R, Bhan AK, Dinarello CA, Springer TA (1986) Induction by IL 1 and interferon-γ: Tissue distribution, biochemistry, and function of a natural adherence molecule (ICAM-1). J Immunol 137:245
13. Ebert T, Bander NH, Finstad CL, Ramsawak RD, Old LJ (1990) Establishment and characterization of human renal cancer and normal kidney cell lines. Cancer Res 50:5531
14. Fady G, Gardner AM, Gera JF, Lichtenstein A (1992) Interferon-induced increase in sensitivity of ovarian cancer targets to lysis by lymphokine-activated killer cells: Selective effects on HER 2/neu-overexpressing cells. Cancer Res 52:764
15. Gerritsen ME, Kelley KA, Ligon G, Perry CA, Shen C, Szczepanski A, Carley WW (1993) Regulation of the expression of intercellular adhesion molecule 1 in cultured human endothelial cells derived from rheumatoid synovium. Arthritis Rheum 36:593
16. Grant AJ, Merchant RE, Hall RE (1989) Interleukin-2 modulates the expression of lymphocyte function-associated antigen-one (LFA-1) and p150,95 during the generation of lymphokine-activated killer (LAK) cells. Immunology 66:117
17. Gregory CD, Murray RJ, Edwards CF, Rickinson AB (1988) Downregulation of cell adhesion molecules LFA-3 and ICAM-1 in Epstein-Barr virus-positive Burkitt's lymphoma

underlies tumor cell escape from virus-specific T cell surveillance. J Exp Med 167:1811
18. Hashimura T, Tubbs RR, Connelly R, Caulfield MJ, Trindade CS, McMahon JT, Galetti TP, Edinger M, Sandberg AA, Cin PD, Sait SJ, Pontes JE (1989) Characterization of two cell lines with distinct phenotypes and genotypes established from a patient with renal cell carcinoma. Cancer Res 49:7064
19. Hildreth JEK, Gotch FM, Hildreth PDK, McMichael AJ (1983) A human lymphocyte-associated antigen involved in cell-mediated lympholysis. Eur J Immunol 13:202
20. Jackson AM, Alexandrov AB, Prescott S, James K, Chisholm, GD (1992) Role of adhesion molecule in lymphokine-activated killer cell killing of bladder cancer cells: further evidence for a third ligand for leukocyte function-associated antigen-1. Immunology 76:286
21. Jevnikar AM, Wuthrich RP, Takei F, Xu H, Brennan DC, Glimcher LH, Rubin-Kelley VE (1990) Differing regulation and function of ICAM-1 and class II antigens on renal tubular cells. Kidney Int 38:417
22. Johnson JP, Stade BG, Holzmann B, Schwable W, Riethmuller G (1989) De novo expression of intercellular-adhesion molecule 1 in melanoma correlates with increased risk of metastasis. Proc Natl Acad Sci USA 86:641
23. Kradin RL, Lazarus DS, Dubinett SM, Gifford J, Grove B, Kurnick JT, Pretter FI, Pinto CE, Davison E, Collahan RJ, Strauss HW (1989) Tumor-infiltrating lymphocytes and interleukin-2 in treatment of advanced cancer. Lancet i:577
24. Kuijpers KC, Kuijpers TW, Zeijlemaker WP, Lucas CJ, van Lier RAW, Miedema F (1990) Analysis of the role of leukocyte function-associated antigen-1 in activation of human influenza virus-specific T cell clones. J Immunol 144:3281
25. Kuppner MC, Van Meir E, Hamou MF, De Tribolet N (1990) Cytokine regulation of intercellular adhesion molecule-1 (ICAM-1) expression on human glioblastoma cells. Clin Exp Immunol 81:142
26. Lanier LL, Loken MR (1984) Human lymphocyte subpopulations identified by using three-color immunofluorescence and flow cytometry analysis: Correlation of Leu-2, Leu-3, Leu-3, Leu-7, Leu-8, and Leu-11 cell surface antigen expression. J Immunol 132:151
27. Makgoba MW, Sanders ME, Luce GEG, Dustin ML, Springer TA, Clark EA, Mannoni P, Shaw S (1988) ICAM-1 a ligand for LFA-1-dependent adhesion of B, T and myeloid cells. Nature 331:86
28. Makgoba MW, Sanders ME, Luce GEG, Gugel EA, Dustin ML, Springer TA, Shaw S (1988) Functional evidence that intercellular adhesion molecule-1 (ICAM-1) is a ligand for LFA-1-dependent adhesion in T cell-mediated cytotoxicity. Eur J Immunol 18:637
29. Myers CL, Desai SN, Schembri-King J, Letts GL, Wallace RW (1992) Discriminatory effects of protein kinase inhibitors and calcium ionophore on endothelial ICAM-1 induction. Am J Physiol 262:C365
30. Naganuma H, Kiessling R, Patarroyo M, Hansson M, Handgretinger R, Gronberg A (1991) Increased susceptibility of IFN-γ treated neuroblastoma cells to lysis by lymphokine-activated killer cells: Participation of ICAM-1 induction of target cells. Int J Cancer 47:527
31. Natali P, Nicotra MR, Cavaliere R, Bigotti A, Romano G, Temponi M, Ferrone SD (1990) Differential expression of intercellular adhesion molecule 1 in primary and metastatic melanoma lesion. Cancer Res 50:1271
32. Pandolfi F, Trentin L, Boyle LA, Stamenkovic I, Byers HR, Colvin RB, Kurnik JT (1992) Expression of cell adhesion molecules in human melanoma cell lines and their role in cytotoxicity mediated by tumor-infiltrating lymphocytes. Cancer 69:1165
33. Rosenberg SA, Lotze MT, Muul LM, Chang AE, Avis FP, Leitman S, Linehan WM, Robertson CN, Lee RE, Rubin JT, Seipp CA, Simpson CG, White DE (1987) A progress report on the treatment of 157 patients with advanced cancer using lymphokine-activated killer cells and interleukin-2 or high dose interleukin-2 alone. N Engl J Med 316:889
34. Rothlein R, Czajkowski M, O'Neill MM, Marlin SD, Mainolfi E, Merluzzi VJ (1988) Induction of intercellular adhesion molecule 1 on primary and continuous cell lines by pro-inflammatory cytokines. Regulation by pharmacologic agents and neutralizing antibodies. J Immunol 141:1665
35. Sanchez-Madrid F, Krensky AM, Ware CF, Robbins E, Strominger JL, Burakoff, SJ, Springer TA (1982) Three distinct antigens associated with human T-lymphocyte-mediated cytolysis: LFA-1, LFA-2, and LFA-3. Proc Natl Acad Sci USA 79:7489
36. Shaw G, Kamen R (1986) A conserved AU sequence from the 3′ untranslated region of GM-CSF mRNA mediates selective mRNA degradation. Cell 46:659
37. Siu G, Hedrick SM, Brian AA (1989) Isolation of the murine intercellular adhesion molecule 1 (ICAM-1) gene. ICAM-1 enhances antigen-specific T cell activation. J. Immunol 143:3813
38. Springer TA (1990) Adhesion receptors of the immune system. Nature 346:425
39. Stauder R, Greil R, Schulz TF, Thaler J, Gattringer C, Radaskiewicz T, Dierich MP, Huber H (1989) Expression of leukocyte function-associated antigen-1 and 7F7-antigen, an adhesion molecule related to intercellular adhesion molecule-1 (ICAM-1) in non-Hodgkin lymphomas and leukaemias: Possible influence on growth pattern and leukaemic behaviour. Clin Exp Immunol 77:234
40. Stauton DE, Marlin SD, Stratowa C, Dustin ML, Springer TA (1988) Primary structure of ICAM-1 demonstrates interaction between members of the immunoglobulin and integrin supergene families. Cell 52:925
41. Tanabe K, Alexander JP, Connelly R, Edinger M, Tubbs RR, Finke JH, Novick AC, Klein EA (1996) The expression of CD44 and CD54 by renal cell carcinoma and their role in cytolysis mediated by autologous tumor infiltrating lymphocytes (TIL) and in IL2 dependent TIL proliferation. (submitted)
42. Tomita Y, Nishiyama T, Watanabe H, Fujiwara M, Sato S (1990) Expression of intercellular adhesion molecule-1 (ICAM-1) on renal-cell cancer: Possible significance in host immune responses. Int J Cancer 46:1001
43. Vanky F, Wang P, Patarroyo M, Klein E (1990) Expression of the adhesion molecule ICAM-1 and major histocompatibility complex class I antigens on human tumor cells is required for their interaction with autologous lymphocytes in vitro. Cancer Immunol Immunother 31:19
44. Vogetseder W, Feichtinger H, Schulz TF, Schwaeble W, Tabaczewski P, Mitterer M, Bock G, Marth C, Dapunt O, Mikuz G, Dierich MP (1989) Expression of 7F7-antigen, a human adhesion molecule identical to intercellular adhesion molecule-1 (ICAM-1) in human carcinomas and their stromal fibroblasts. Int J Cancer 43:768
45. Wertheimer SJ, Myers CL, Wallace RW, Parks TP (1992) Intercellular adhesion molecule-1 gene expression in human endothelial cells. Differential regulation by tumor necrosis factor-a and phorbol myristate acetate. J Biol Chem 267: 12030
46. Wilson T, Treisman R (1988) Removal of poly (A) and consequent degradation of c-*fos* mRNA facilitated by 3′AU-rich sequences. Nature 336:396
47. Wuthrich RP (1992) Tumor necrosis factor-a-and interleukin-1-stimulated intercellular adhesion molecule-1 expression by murine renal tubular epithelial cells is transcriptionally regulated and involves protein kinase C. Renal Physiol Biochem 15:302