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Stimulation of intercellular adhesion molecule-1 (ICAM-1) antigen expression and shedding by interferon-γ and phorbol ester in human renal carcinoma cell cultures: relation to peripheral blood mononuclear cell adhesion

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Abstract In the present study we investigated the effect of interferon-γ (IFN-γ) and phorbol-12-myristate 13 acetate (PMA) on intercellular adhesion molecule-1 (ICAM-1) antigen expression and shedding in human renal carcinoma cell cultures. We also examined the functional consequences of ICAM-1 antigen expression and soluble ICAM-1 molecules on the adhesion of peripheral blood mononuclear cells (PBMC). Incubation of the human renal carcinoma cell line CaKi-1 with IFN-γ or PMA enhanced ICAM-1 antigen expression. The calcium ionophore, 4-bromo-calcium ionophore A23187 (Bromo-A23187) significantly enhanced the IFN-γ and PMA effect. Soluble ICAM-1 (sICAM-1) was detected in the supernatants of stimulated but not unstimulated cultures, and correlated significantly with cellular expression. Using ^{51}Cr-labelled peripheral blood mononuclear cells in a cell adhesion assay, we demonstrated increased adhesion in IFN-γ-treated CaKi-1 cultures, which was augmented by Bromo-A23187. This adhesion was blocked by preincubation of CaKi-1 cells with monoclonal antibody against ICAM-1 or by preincubation of PBMC with either monoclonal antibody against leucocyte function associated antigen-1 α (LFA-1α), a major receptor for ICAM-1, supernatants from treated cultures or purified sICAM-1 molecules. Thus, shedding of ICAM-1 may play a role during the escape from immunosurveillance by renal carcinoma cells.

Key words ICAM-1 · Interferon-γ · Phorbol ester · Renal cell carcinoma · Adhesion

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Intercellular adhesion molecule-1 (ICAM-1) is a cell-surface-related glycoprotein of the immunoglobulin superfamily and ligand for leucocyte-function-associated antigen-1 (LFA-1). While LFA-1 is constitutively expressed on all leucocytes, ICAM-1 is inducible on a wide range of normal and neoplastic cells by inflammatory cytokines such as interleukin-1β (IL-1β), tumour necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) [18]. The coupling between ICAM-1 and LFA-1 plays an essential role in the initiation of a specific immune response, since antigen-independent interactions between adhesion molecules are required as well as activation through antigen-specific receptors on lymphocytes [14, 21].

The role of ICAM-1 during antitumoral immunity remains to be defined. Recent reports suggest that ICAM-1 together with major histocompatibility complex (MHC) class I antigen expression on human tumour cells is required for their interaction with autologous lymphocytes [24]. Tomita et al. [22] have found that ICAM-1 expression on human renal carcinoma cells might augment the host immune reaction. In contrast, upregulation of ICAM-1 expression on melanoma cells has been shown to correlate with a greater risk of metastasis [11]. The mechanism(s) by which ICAM-1 expression may enhance metastatic potential is unknown. One explanation may reside in the fact that soluble ICAM-1 molecules (sICAM-1) shed by tumour cells may allow them to escape immunosurveillance by blocking counter receptor LFA-1 sites on leucocytes and thereby inhibiting immune recognition.

We have previously shown that in the human renal carcinoma cell line CaKi-1, IFN-γ stimulates ICAM-1 antigen expression possibly by a calcium-dependent signal transduction pathway, while the phorbol ester, phorbol-12-myristate 13 acetate (PMA), enhances ICAM-1 antigen expression through activation of the calcium/phospholipid-dependent protein kinase C (PKC) [8]. However, the effect of IFN-γ and PMA on sICAM-1 shedding and ICAM-1-dependent cell adhesion was not examined.

Hence, the purpose of this study was to correlate IFN-γ and PMA-induced ICAM-1 antigen expression on CaKi-1

cells with ICAM-1 shedding into the cell culture supernatants. Furthermore, we wished to investigate the influence of IFN-γ and PMA on ICAM-1/LFA-1-mediated mononuclear cell-CaKi-1 cell adhesion, since this adhesion system could play a role in the differential susceptibility of renal carcinoma cells to immunotherapy.

Materials and methods

Reagents

Human recombinant IFN-γ ($1–5 \times 10^7$ units/mg protein) designated IFN-γ 4A was purchased from Amersham (Amersham International, Denmark). PMA and the calcium ionophore 4-bromocalcium ionophore A23187 (Bromo-A23187) were obtained from Sigma (St. Louis, Mo., USA). IFN-γ was stored at 2–4°C diluted to 10^4 U/ml in McCoy's 5A medium containing 10% fetal bovine serum (FBS) (Gibco, Paisley, UK). PMA and Bromo-A23187 were dissolved in dimethylsulfoxide (DMSO) and 1 mg/ml stock solutions were stored at –80°C. The final concentration of DMSO did not exceed 0.3%, and cultures containing appropriate quantities of diluents were run in parallel to control for solvent effects.

Cell cultures

The human renal carcinoma cell line, CaKi-1 was kindly provided by Dr. J. J. Fogh (Novo Nordisk, Gentofte, Denmark) and was originally isolated and characterized by the late Dr. J. Fogh, (Memorial Sloan Kettering, Rye, N.Y., USA) [5]. They were maintained in McCoy's 5A medium (Gibco), supplemented with 10% FBS (Gibco), 2 mM glutamine (Gibco) and 100 IU/ml penicillin/streptomycin (Gibco). Cultures were incubated at 37°C in a 95% air, 5% CO_2 humid incubator.

Flow cytometric analysis of ICAM-1 antigen

At confluent monolayer in 75 cm^2 tissue flasks (Nunc, Roskilde, Denmark), CaKi-1 cells were obtained for subculture by addition of 0.15% trypsin (Gibco) in calcium-free phosphate buffer (pH 7.2), blocked by McCoy's 5A with 10% FBS and the detached cells were centrifuged and resuspended in fresh medium. A volume of 1 ml of a 10^7 cells/ml suspension was seeded in 25 cm^2 tissue flasks (Nunc). After 48 h of culture, cells were treated with IFN-γ, PMA and Bromo-A23187 in the indicated combinations, concentrations and time courses. After stimulation cells were washed twice with Hank's balanced salt solution without Ca^{2+}/Mg^{2+}/phenol red (HBSS) (Gibco)/1% bovine serum albumin (BSA) (Sigma). After washing, cells were detached by incubation with 2 ml, 1 mM EDTA in HBSS/1% BSA for 30 min at 37°C. One hundred microlitres of the cell suspension was transferred to 12 × 75 mm polystyrene tubes (Falcon, Becton Dickinson) and incubated at 4°C in the dark for 30 min with fluorescein isothiocyanate (FITC) conjugated monoclonal anti ICAM-1, clone 84H10 [13] (Immunotech, Marseilles, France), diluted 10 µl in 10 µl HBSS. After staining, cells were washed twice with HBSS/1% BSA by centrifugation at 300 *g* for 5 min and resuspended in 500 µl fixation buffer (HBSS with 1% paraformaldehyde, pH 7.4). Analysis of fluorescence was performed on a FACScan (Becton Dickinson, Mountain View, Calif., USA). The background number of fluorescent cells (no relevant monoclonal antibody) was generally adjusted to less than 1% and the relative mean fluorescense intensity (MFI) of positive cells was measured. The irrelevant mouse IgG1 FITC conjugated antibody X927 (DAKO, Denmark) was used as a negative control.

Assay of soluble ICAM-1 molecules

CaKi-1 cells were obtained for subculture as described for the FACS analysis (see above) and 1 ml of a 10^7 cells/ml suspension was seeded in 25 cm^2 tissue flasks. After 48 h of culture, cells were stimulated with IFN-γ, PMA and Bromo-A23187 for 24–72 h as indicated. Culture supernatants were harvested and levels of sICAM-1 were measured with a commercial ELISA kit with an assay sensitivity of < 0.35 ng/ml (British Bio-technology Products, Oxford, UK, version 2).

Isolation and ^{51}Cr-labelling of human peripheral blood mononuclear cells

Human peripheral blood mononuclear cells (PBMC) were isolated from venous blood of healthy donors by Ficoll metrizoate (Lymphoprep; Nycomed, W. Midlands, UK) density gradient centrifugation [3] of 10 ml heparinized blood. The PBMC were then resuspended in PBMC medium composed of RPMI-1640 (Northumbria Biologicals, Northumberland, UK) supplemented with 10% FBS and penicillin/streptomycin 100 IU/ml. For ^{51}Cr-labelling, 1×10^7 cells/ml were incubated for 90 min with 200 µCi/ml of Na_2 ^{51}Cr O_4 (Amersham International, Denmark), then washed four times by centrifugation in PBMC medium.

PBMC – CaKi-1 adhesion assay

CaKi-1 cells were plated at 5×10^4 cells/well in flat-bottomed 96-well plates (Falcon) and grown to subconfluence (48 h) in McCoy's 5A medium containing 10% FBS, 2 mM glutamine and 100 IU/ml penicillin/streptomycin. IFN-γ and Bromo-A23187 were added for 48 h in the indicated combinations. Binding of PBMC to unstimulated and stimulated CaKi-1 cells was measured as described previously [10]. Briefly, CaKi-1 monolayers were washed three times with warm PBMC medium, 1×10^5 ^{51}Cr-labelled PBMC were added in 0.2 ml of PBMC medium and the plates were incubated for 60 min at 37°C. The wells were then washed gently three times with warm PBMC medium to remove non-adherent PBMC and the remaining adherent PBMC were lysed by addition of 0.2 ml Triton X-100 (Sigma). Radioactivity of the lysate was calculated using a gamma counter. The percentage of adherent PBMC was calculated by the following formula:

$$\% \text{ adhesion of PBMC} = \frac{\text{cpm in 0.2 ml lysate}}{\text{cpm in 0.2 ml original cell suspension}} \times 100 \quad [10]$$

In some experiments, CaKi-1 cells or PBMC were pretreated with anti-ICAM-1 (1:5) (Immunotech), anti-LFA-1α (1:5) (Immunotech.), anti-human leucocyte antigen (HLA)-A, B, C (1:5) (Sera-lab.) monoclonal antibodies, increasing doses of purified sICAM-1 (British Bio-technology Products) or culture supernatants for 30 min at 37°C prior to performing the adhesion assay. In a recent study using an indirect immunoperoxidase technique, Heufelder and Bahn [9] demonstrated that monoclonal mouse anti-ICAM-1 antibody can inhibit PBMC adhesion to orbital fibroblasts at 10 times immunoperoxidase staining concentrations. We have previously shown that the end-point immunoperoxidase staining concentration for anti-ICAM-1, clone 84H10 in CaKi-1 cell cultures is 1:50 [8]. Hence, specific antibodies for the inhibition experiments were used at a dilution of 1:5, representing a value above the concentration used for the direct immunofluorescence analysis (see above) and 10 times the concentration used for indirect immunoperoxidase staining [8].

Statistical analysis

All results represent mean ± 1SD of three different experiments. Data were analysed by student's *t*-test. $P < 0.05$ was considered to represent statistical significance.

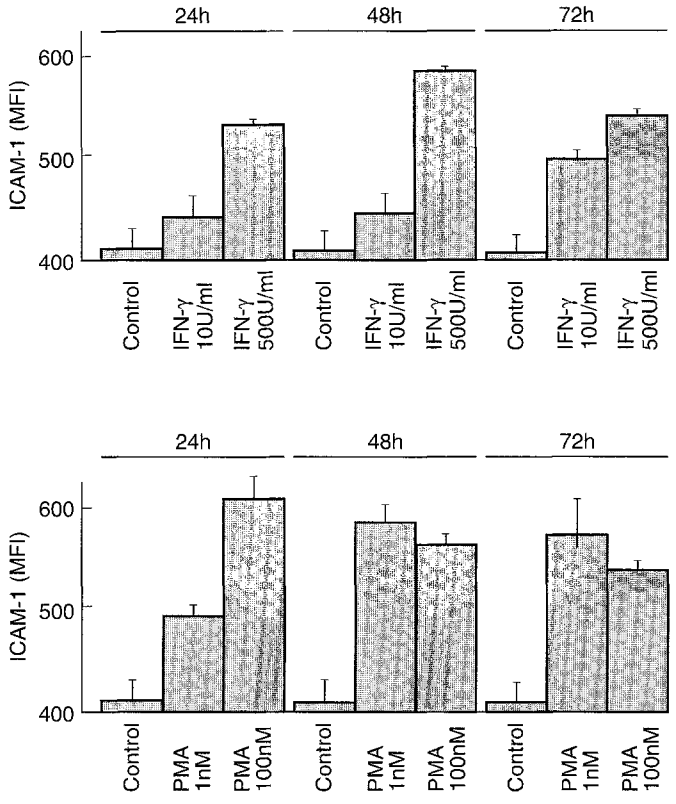

Fig. 1 Effect of IFN-γ (*top*) and PMA (*bottom*) on ICAM-1 antigen expression. CaKi-1 cells were incubated with minimal and maximal stimulatory doses of IFN-γ or PMA and analysed by FACS. ICAM-1 antigen expression was significantly enhanced by 500 U/ml IFN-γ, 1 nM PMA and 100 nM PMA after 24, 48 and 72 h while 10 U/ml IFN-γ led to significant enhancement above control at 72 h. Data represent mean ± 1SD of 3 experiments

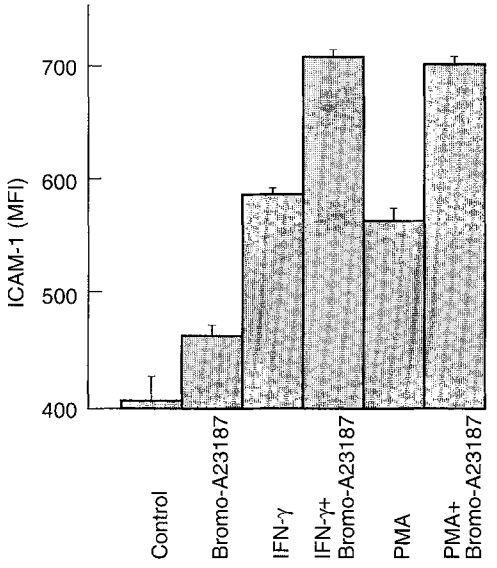

Fig. 2 Effect of Bromo-A23187 (0.5 μM) on IFN-γ (500 U/ml) and PMA (100 nM) induced ICAM-1 antigen expression. CaKi-1 cells were incubated for 48 h and analysed by FACS. Bromo-A23187 significantly enhanced IFN-γ and PMA-induced ICAM-1 antigen expression. Data represent mean ± 1SD of 3 experiments

Pearson's correlation coefficient (r) was used to determine the degree of correlation between ICAM-1 antigen expression (MFI) as measured by FACS analysis and shed sICAM-1 (ng/ml) as measured by ELISA.

Results

Effect of IFN-γ, PMA and Bromo-A23187 on ICAM-1 MFI

CaKi-1 cells were incubated for 24, 48 and 72 h with IFN-γ (10 U/ml and 500 U/ml) and PMA (1 nM and 100 nM). These doses were chosen on the basis of previous studies, representing minimal and maximal stimulatory doses of IFN-γ and PMA for ICAM-1 antigen induction as analysed by immunocytochemistry [8]. A dose-dependent induction of ICAM-1 antigen was seen after 72 h of IFN-γ treatment (Fig. 1, top). At 24 and 48 h, 500 U/ml IFN-γ significantly increased ICAM-1 antigen induction, while 10 U/ml had no significant effect. PMA increased ICAM-1 antigen in a dose-dependent manner at 24 h, while there were no significant differences between 1 nM and 100 nM PMA at 48 and 72 h (Fig. 1, bottom). Since both IFN-γ and PMA signal transduction in CaKi-1 cells may be dependent upon a rise in intracellular calcium ion [8], we incubated CaKi-1 cells for 48 h with Bromo-A23187 (0.5 μM), a carboxylic acid antibiotic which increases intracellular calcium ions by facilitating their transport across biological membranes [12]. Bromo-A23187 significantly increased ICAM-1 antigen expression above control levels and also significantly enhanced the effect of IFN-γ (500 U/ml) and PMA (100 nM) for 48 h as compared to either agent alone (Fig. 2).

Effect of IFN-γ, PMA and Bromo-A23187 on the shedding of sICAM-1

The concentration of sICAM-1 was not detectable (ND) in unstimulated cultures grown for 24–72 h (Fig. 3, top). IFN-γ 10 U/ml for 24 h was able to raise sICAM-1 (11 ± 4 ng/ml) above control levels. Increasing the IFN-γ dose to 500 U/ml had no significant effect until 72 h, when sICAM-1 was raised to 23 ± 3 ng/ml. PMA 1 nM for 24 h also significantly enhanced sICAM-1 (12 ± 6 ng/ml) above control levels (Fig. 3, bottom). Increasing the PMA dose to 100 nM significantly affected ICAM-1 shedding after 48 h with maximal levels reaching 33 ± 9 ng/ml at 72 h. Furthermore, Bromo-A23187 (0.5 μM) for 48 h significantly raised sICAM-1 (10 ± 2 ng/ml) as compared to control levels. Combining Bromo-A23187 (0.5 μM) with IFN-γ (500 U/ml) or PMA (100 nM) for 48 h resulted in a significant increase in sICAM-1 from 19 ± 3 ng/ml and 26 ± 4 ng/ml for IFN-γ and PMA to 32 ± 3 ng/ml and 50 ± 5 ng/ml for IFN-γ plus Bromo-A23187 and PMA plus Bromo-A23187, respectively (Fig. 4). Finally, there was a significant correlation between ICAM-1 antigen expression as

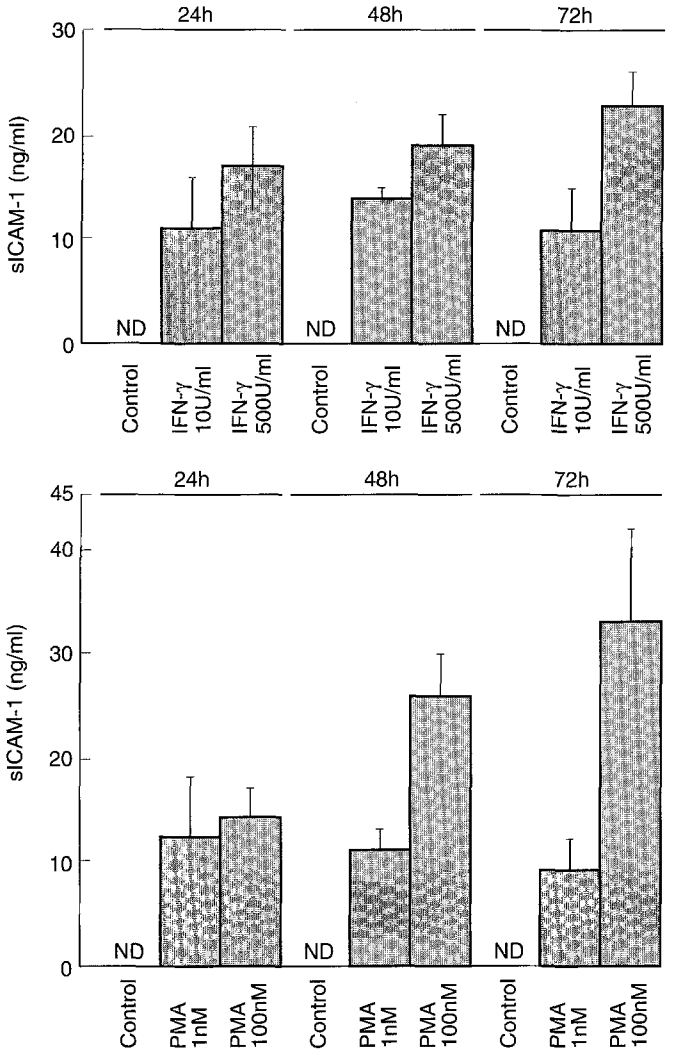

Fig. 3 Effect of IFN-γ (*top*) and PMA (*bottom*) on the shedding of sICAM-1. CaKi-1 cells were incubated with minimal and maximal stimulatory doses of IFN-γ or PMA and sICAM-1 in culture supernatants was analysed by ELISA. sICAM-1 was not detectable (*ND*) in control supernatants, while IFN-γ and PMA significantly enhanced the release of sICAM-1 after 24, 48 and 72 h. Data represent mean ± 1SD of 3 experiments

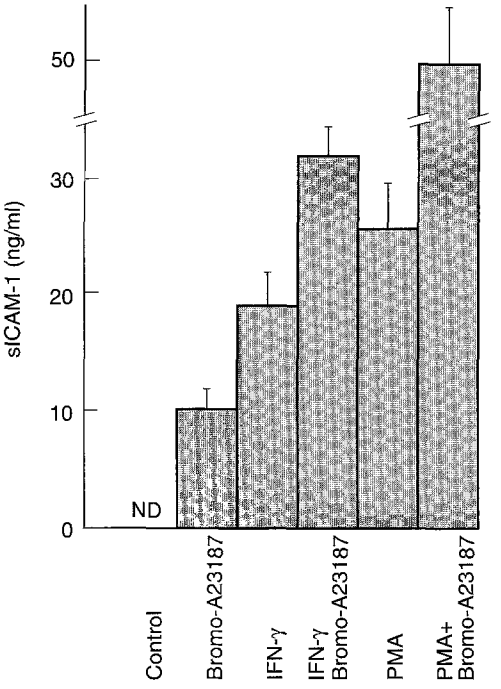

Fig. 4 Effect of Bromo-A23187 (0.5 μM) on IFN-γ (500 U/ml) and PMA (100 nM) induced shedding of sICAM-1. CaKi-1 cells were incubated for 48 h and sICAM-1 in culture supernatants was analysed by ELISA. Bromo-A23187 significantly enhanced IFN-γ and PMA induced shedding. Data represent mean ± 1SD of 3 experiments

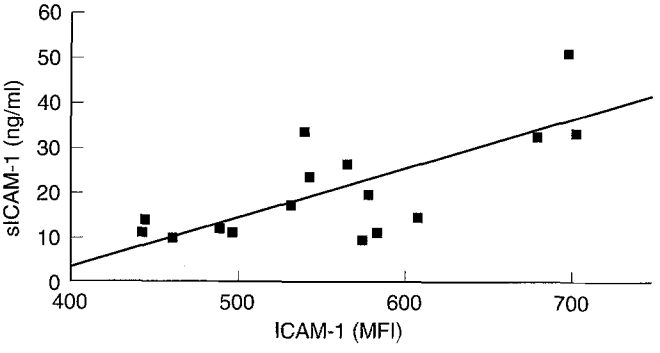

Fig. 5 Relation between ICAM-1 antigen expression (MFI) and shedding of sICAM-1 (sICAM-1, ng/ml). The regression line is sICAM-1 (ng/ml) = 0.1 (ICAM-1 MFI) −37.7; and $r = 0.76$, $P = 0.0004$

analysed by FACS and shed sICAM-1 ($r = 0.76$, $P = 0.0004$) (Fig. 5).

Effect of IFN-γ on PBMC – CaKi-1 adhesion and modulation by monoclonal antibodies, supernatant transfer and purified sICAM-1

Adhesion of PBMC to unstimulated control CaKi-1 cultures was 492 ± 41 cpm. Adhesion was significantly enhanced by stimulation of CaKi-1 cells with Bromo-A23187 0.5 μM for 48 h (1189 ± 88 cpm), IFN-γ 500 U/ml for 48 h (2049 ± 196 cpm) or both (2838 ± 427 cpm) (Fig. 6). To investigate the contribution of different surface molecules to the adhesion between PBMC and CaKi-1 cells, we stimulated CaKi-1 monolayers with IFN-γ 500 U/ml for 48 h and pretreated either CaKi-1 monolayers or PBMC with monoclonal antibodies directed against ICAM-1, LFA-1α or HLA-A,B,C, diluted 1:5 each. PBMC adhesion to IFN-γ stimulated (500 U/ml for 48 h) but non-pretreated CaKi-1 cells was considered to be the control level (100%) corresponding to 2049 ± 196 cpm and percentage adhesion of PBMC was calculated as a percentage thereof [9]. Preincubation of CaKi-1 cells with anti-ICAM-1 significantly reduced adhesion from the control level to 760 ± 108 cpm. Also, a significant reduction in

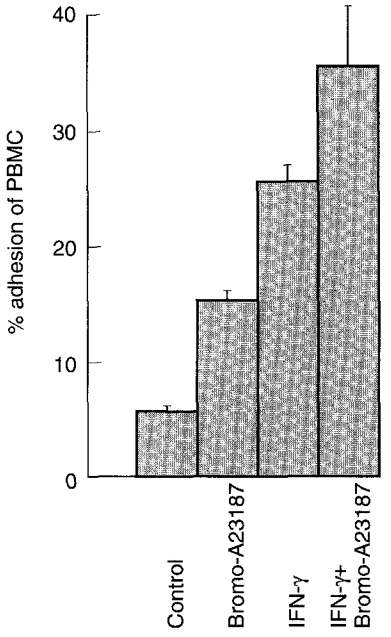

Fig. 6 Effect of Bromo-A23187 (0.5 μM) and IFN-γ (500 U/ml) alone and in combination on percentage adhesion of PBMC. CaKi-1 cells were stimulated with the drugs for 48 h and the percentage adhesion of ^{51}Cr-labelled PBMC was determined. Bromo-A23187 and IFN-γ alone or in combination significantly enhanced adhesion above control levels. Data represent mean ± 1SD of 3 experiments

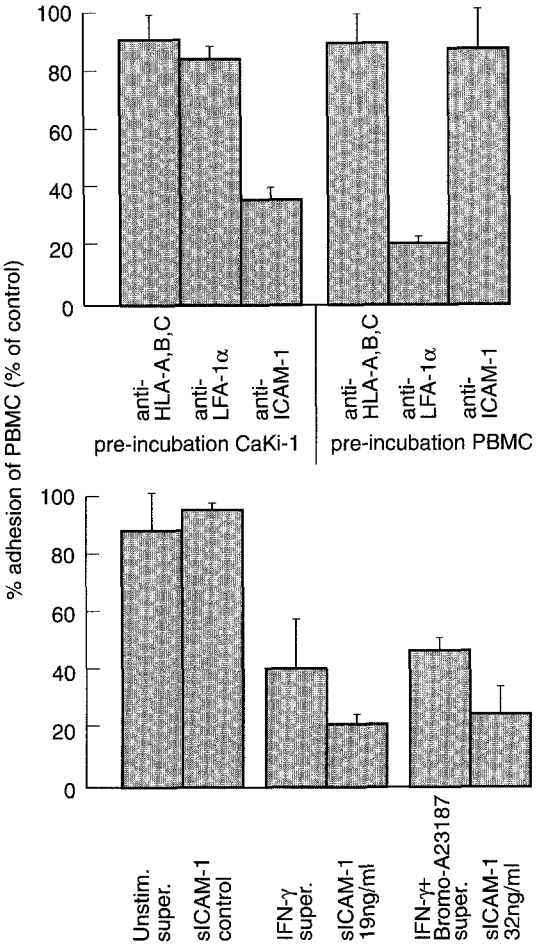

Fig. 7 Effect of monoclonal antibodies, supernatants and purified sICAM-1 on percentage adhesion of PBMC (% of control). PBMC adhesion to IFN-γ stimulated (500 U/ml for 48 h) CaKi-1 cells was considered as the control value (100%). Preincubating CaKi-1 cells with anti-ICAM-1 or PBMC with anti-LFA-1α significantly reduced adhesion (*top*). Preincubating PBMC with 19 ng/ml or 32 ng/ml purified sICAM-1 or their equivalent supernatants (IFN-γ super, IFN-γ plus Bromo-A23187 super) was approximately equally effective in reducing adhesion (*bottom*). Data represent mean ± 1SD of 3 experiments

adhesion was obtained by preincubating PBMC with anti-LFA-1α (393 ± 97 cpm). Preincubation of CaKi-1 with anti-LFA-1α, PBMC with anti-ICAM-1 or either with anti-HLA-A,B,C had no effect (Fig. 7, top).

In order to evaluate the effect of shed sICAM-1 on adhesion of PBMC to IFN-γ stimulated (500 U/ml for 48 h) CaKi-1 cells (control level, 100% equal to 2049 ± 196 cpm), PBMC were pretreated with culture supernatants from CaKi-1 cells or purified sICAM-1. Hence, supernatants from unstimulated cultures (unstim super) containing non-detectable sICAM-1 were compared to 0 ng/ml purified sICAM-1 (vehicle alone); supernatants from cultures stimulated with IFN-γ 500 U/ml for 48 h (IFN-γ super) containing 19 ± 3 ng/ml sICAM-1 were compared to 19 ng/ml purified sICAM-1 and finally supernatants from cultures stimulated with IFN-γ 500 U/ml plus Bromo-A23187 0.5 μM for 48 h (IFN-γ + Bromo-A23187 super) containing 32 ± 3 ng/ml sICAM-1 were compared to 32 ng/ml purified sICAM-1. Supernatants from cultures stimulated with IFN-γ significantly reduced adhesion of PBMC to 779 ± 257 cpm, while supernatants from cultures stimulated with IFN-γ plus Bromo-A23187 reduced adhesion to 985 ± 165 cpm. Supernatants from unstimulated cultures had no effect (Fig. 7, bottom). There was no significant difference between supernatants from IFN-γ or IFN-γ plus Bromo-A23187 treated cultures. Purified sICAM-1 at 19 ng/ml and 32 ng/ml significantly inhibited adhesion of PBMC to 448 ± 127 cpm and 501 ± 113 cpm, respectively. Vehicle without sICAM-1 had no effect (Fig. 7, bottom). However, 32 ng/ml sICAM-1 had a slightly greater effect than its corresponding supernatant (IFN-γ + Bromo-A23187 super). This was not the case for 19 ng/ml sICAM-1.

Discussion

Numerous studies have described the effect of IFN-γ and phorbol esters on ICAM-1 antigen expression on normal and neoplastic cells [7, 8, 17, 18]. However, only a few recent reports have described the co-existence of a soluble variant of ICAM-1 in the supernatants from tumour cells in culture [6, 19, 20]. Since the shedding of sICAM-1 from tumour cells may play a role in the differential susceptibility to immunotherapy by interfering with effector cell adhesion [4], we investigated the possible relation between

IFN-γ and PMA-stimulated ICAM-1 antigen expression and sICAM-1 shedding by CaKi-1 cells. We also examined the effect of sICAM-1 on PBMC-CaKi-1 cell adhesion.

Our data showed a significant increase in ICAM-1 antigen expression analysed by FACS after stimulation with IFN-γ or PMA (Fig. 1). We have previously found that both IFN-γ and PMA may be dependent upon intracellular calcium during ICAM-1 antigen induction in CaKi-1 cells [8]. In agreement with these studies, the calcium ionophore Bromo-A23187 was able to enhance the effect of IFN-γ and PMA on ICAM-1 antigen upregulation (Fig. 2). Using an ELISA which is specific for human ICAM-1, we have demonstrated that cellular expression and shedding of ICAM-1 correlates closely in CaKi-1 cells (Fig. 5). Since the main counterligand for ICAM-1 is LFA-1 expressed on all leucocytes, and since the significance of ICAM-1 molecule shedding is not clear, we chose to compare ICAM-1/LFA-1α-mediated PBMC-CaKi-1 cell adhesion to shed sICAM-1. Corresponding to the ICAM-1 expression analysed by FACS, PBMC attachment to CaKi-1 cells was significantly increased above control levels by Bromo-A23187, IFN-γ and their combination (Fig. 6). PMA was not used to induce ICAM-1 antigen in the adhesion assay since phorbol esters can enhance adhesion, irrespective of ICAM-1 antigen expression by activating PBMC [9]. The significant reduction in PBMC adhesion following preincubation of IFN-γ stimulated CaKi-1 cells with anti-ICAM-1 confirms that the induced ICAM-1 molecule was functionally active [16] (Fig. 7, top). The partial reduction of PBMC adhesion to IFN-γ stimulated CaKi-1 cells by preincubation of PBMC with anti-LFA-1α suggests that the alpha-chain of LFA-1 is of importance in the attachment of PBMC to ICAM-1 on CaKi-1 cells [23] (Fig. 7, top). Adhesive interactions between CD8-positive lymphocytes (suppressor/cytotoxic T cells) and the α 3 domain of class I HLA has been described [21]. Vanky et al. [24] have shown that both ICAM-1 and MHC class I antigen expression on tumour cells is required for their interaction with autologous lymphocytes. However, adhesion was not affected by anti-HLA-A,B,C preincubation of either CaKi-1 cells or PBMC. Finally, PBMC adhesion was markedly reduced by preincubating PBMC with purified sICAM-1 or supernatants from stimulated CaKi-1 cultures containing shed sICAM-1 (Fig. 7, bottom). These results support the hypothesis that sICAM-1 blocks PBMC adhesion by binding to PBMC and that sICAM-1 shed, by CaKi-1 cells, may be functionally active in this respect. Purified SICAM-1 (32 ng/ml) was more effective than IFN-γ + Bromo-A23187 super, with equal amounts of shed sICAM-1, regarding inhibition of PBMC adhesion. The discrepancy was, however, small and may not reflect a physiologically relevant phenomenon.

The effect of cytokines on ICAM-1 antigen expression and release has been examined in other tumour cell types. In human melanoma cell lines IFN-γ and TNF-α-induced ICAM-1 antigen expression correlates closely with release of sICAM-1 as determined by sandwich ELISA [19]. In human pancreatic carcinoma cells sICAM-1 is shed from those cell lines which constitutively express ICAM-1 antigen on their cell surface. TNF-α can enhance sICAM-1 shedding by approximately 3-fold [20]. However, the significance of the shed sICAM-1 regarding adhesion was not investigated in these two studies. Our data suggest that high local concentrations of sICAM-1 could act to block immune recognition by leucocytes. Evidence to support this possibility has come from Becker et al. [2], who have demonstrated that sICAM-1 shed from human melanoma cells can block natural or lymphokine activated killer cell – mediated cytotoxicity. The total amount of sICAM-1 shed by CaKi-1 cells was approximately the same as detected in the melanoma cell supernatants [2]. Besides having a functional role, sICAM-1 may represent a potential marker for tumour diagnosis and progression, since recent clinical studies have detected elevated serum levels of circulating sICAM-1 in patients with different malignancies as compared to normal individuals [1, 15].

In conclusion, our findings suggest that IFN-γ and PMA induces ICAM-1 antigen expression in Caki-1 cells, which correlates closely with the shedding of sICAM-1. This effect can be enhanced by Bromo-A23187 in agreement with the possible dependence of IFN-γ and PMA signal transduction on intracellular Ca^{2+} transients. The induced ICAM-1 antigen expression is functionally active with respect to PBMC-CaKi-1 cell adhesion, while the shed sICAM-1 can block this function. Although this indicates that local sICAM-1 molecules can block immune recognition by leucocytes, further studies are needed to determine the significance of shed sICAM-1 on tumour metastasis.

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