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ORIGINAL ARTICLE

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Soluble ICAM-1 in breast cancer: clinical significance and biological implications

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Abstract Objectives: In previous experiments, we demonstrated a decreased expression of intercellular adhesion molecule 1 (ICAM-1) on both tumour cells and antigen-presenting cells derived from patients with breast cancer, resulting in an abrogation of antigen presentation and tumour cell lysis. Recently, increased levels of a soluble isoform of ICAM-1 (sICAM-1) have been detected in the sera of breast cancer patients. The present investigation was performed in order to investigate the biological relevance of serum concentrations and the effects of sICAM-1 in patients with breast cancer. **Patients and methods:** sICAM-1 was determined using a sandwich enzyme immunoassay on sera from 88 patients with various stages of breast cancer and correlated with clinical parameters. The effect of sICAM-1 present in the sera of patients with breast cancer upon unspecific and anti-Her-2/neu antibody-mediated cytotoxicity (ADCC), as well as upon antigen presentation, was determined using a ^{51}Cr-release assay and [^{3}H]-thymidine-uptake of T cells after co-incubation with tetanus-toxoid-pulsed antigen-presenting cells. **Results:** In patients with early breast cancer, serum levels of sICAM-1 were significantly lower compared to patients with metastatic disease, but did not correlate with usual clinical parameters. In patients with metastatic breast cancer, a significant correlation of sICAM-1 with tumour markers CEA and CA 15-3 was observed. No influence of sICAM-1 upon unspecific cytotoxicity, ADCC, or the ability to present antigen was observed. **Discussion:** The origin of sICAM-1 in the sera of patients with breast cancer remains unknown. In contrast to its membrane-bound isoform, sICAM-1 was increased in the sera of patients with various stages of breast cancer, but its presence did not influence unspecific cytotoxicity, ADCC, or antigen-induced T cell proliferation.

Keywords sICAM-1 · Breast cancer · ADCC · Antigen presentation

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Introduction

Initial steps in the specific activation of the immune system function via the interaction of cell populations and depend upon the presence of various molecules. These molecules, including antigens CD80, CD86 and CD28, as well as CD152, are present on antigen-presenting cells (APC) and T cells, respectively, and act as costimulatory signals. An appropriate immune response can only develop in the presence of these molecules, whereas their absence results in anergy [41]. Moreover, similar mechanisms are also operational in nonspecific immune mechanisms, including cell-mediated lysis of target cells. Intercellular adhesion molecule-1 (ICAM-1), which represents a glycoprotein from the immunoglobulin supergene family, has been shown to possess both abilities, as it acts both as a costimulatory molecule within the context of APC-T cell interaction [13] and as a target-cell-associated ligand of lymphocyte-function-associated antigen 1 (LFA-1, CD11a/CD18) present on natural killer cells (NK) [10], lymphokine-activated killer cells (LAK) and cytotoxic T lymphocytes (CTL) [21, 23], respectively. Moreover, LFA-1-dependent interactions are partly involved in antibody-dependent cytotoxicity, and leukocyte adhesion and extravasation across the vascular endothelium [11, 26, 46].

The biological importance of ICAM-1 in the context of breast cancer has been suggested by previous investigations, which showed defective regulation of ICAM-1 not only on tumour cells [8], but also on APC derived from patients with cancer, resulting in both an impaired recognition and attack of malignant target cells by immunological effector cells and in a decreased ability to present alloantigen [44]. These results were not only corroborated by findings from other laboratories, but further expanded to clinical analyses, which demonstrated that an increased expression of ICAM-1 by breast cancer cells from tumour specimens was associated with lower tumour stage, as well as histopathological nuclear grading, and with longer relapse-free period and overall survival of patients [5, 8, 27]. According to the various mentioned roles of ICAM-1, the latter observations were interpreted to the extent that a lack of ICAM-1 expression could contribute to metastatic spread by either lack of tumour cell adhesion or escape from immune surveillance [5, 8, 27].

In continuation of the latter observations, it is interesting to note that the existence of a soluble isoform of ICAM-1 (sICAM-1) has been reported, which was found to be elevated in the sera of patients with various inflammatory and malignant disorders, including breast cancer, and represented an adverse prognostic parameter predicting a significantly poorer overall survival and lower response to cytotoxic or endocrine therapy [4, 17, 18, 19, 29, 30, 36, 38, 40, 47].

Within this context, both expression of an alternatively spliced messenger RNA form coding for sICAM-1 and, more likely, distinct mechanisms of proteolytic cleavage of ICAM-1, resulting in the release of sICAM-1, have been postulated to occur [9, 42, 43]. Since sICAM-1 possesses most of the extracellular domain of membranous ICAM-1 [9], it is capable of binding to LFA-1 [24, 36], thus potentially interfering with various immunological functions. Furthermore, sICAM-1 has been demonstrated to block lymphocyte attachment to both endothelial cells and ICAM-1-expressing tumour cells, resulting in an abrogation of MHC-restricted and non-MHC-restricted cytolysis of target cells by CTL, NK and LAK [1, 3, 6, 7, 20, 33, 37]. In addition, sICAM-1 has been demonstrated to inhibit proliferation of autoreactive T cells, whereas alloantigen-induced T cell proliferation was found to be enhanced by sICAM-1 [24, 34]. However, within the context of malignant disorders, the role of sICAM-1 upon antigen presentation remains unknown. Since novel active immunotherapeutic strategies, including vaccination protocols [12], essentially rely upon intact ICAM-1-dependent processes of antigen presentation, the understanding of the effects of sICAM-1 upon alloantigen-induced T cell responses is of crucial importance. In addition, the origin and long-term immunological effects of elevated serum levels of sICAM-1 observed in patients with malignant disorders, as well as their impact upon adoptive immunotherapeutic strategies, remain unknown. In an attempt to elucidate whether the decreased expression of ICAM-1 on breast cancer cells and APC found in previous experiments was due to solubilisation of the molecule, sICAM-1 concentrations were assessed in supernatants of breast cancer cell lines and monocytes derived from patients with breast cancer, but were not found to be increased [8, 44].

Consequently, we were interested to verify previous observations of elevated serum levels of sICAM-1 in patients with breast cancer and to investigate the biological consequences of this phenomenon. Recent developments have successfully introduced the antibody Trastuzumab, directed against the Her-2/neu protein, into the treatment of Her-2/neu protein over-expressing breast cancer [28]. As Trastuzumab partly acts via mechanisms known from antibody-dependent cellular cytotoxicity (ADCC), its dependence upon the expression of ICAM-1 and LFA-1 was intriguing to investigate. These assays showed that the sensitivity of tumour cells towards Trastuzumab-mediated ADCC was independent from their surface expression of ICAM-1, but anti-LFA-1 antibodies decreased Trastuzumab-mediated ADCC [11]. Thus, we were interested to investigate whether sICAM-1 interacting with LFA-1 may influence the efficacy of adoptive immunotherapy in patients with breast cancer.

In the present investigation, we report on elevated serum levels of sICAM-1 in patients with various stages of breast cancer and the biological consequences of elevated sICAM-1 in patients and matched healthy controls to elucidate both short term and long term effects.

Patients and methods

Analysis of serum levels of sICAM-1

The sera of 88 patients with a median age of 56.1 years (range 25.9–76.4 years) and histologically verified breast cancer were investigated. Forty-one patients (median age 53.7 years, range 25.9–76.4 years) had stage I to III breast cancer, whereas 47 patients (median age 57.2 years, range 37.3–76.3 years) had stage IV disease. Patients' characteristics are shown in Table 1.

Blood was drawn in the morning, immediately centrifuged and the resulting sera stored at –80°C. In all patients, tumour stage, histopathological grading, oestrogen and progesterone receptor status (performed routinely by ERICA and PgRICA assays), blood biochemistry and current tumour markers CEA and CA 15-3 (performed routinely with Enzymun-Test CEA and Enzymun-Test CA 15-3, respectively; Boehringer-Mannheim, Germany) were recorded. M stage was determined by physical examination, (chest) X-ray, bone scan, sonography and computed tomography. All patients with early breast cancer had already undergone primary surgery and most patients had received different therapeutic regimens (i.e. endocrine therapy, cytotoxic chemotherapy, radiotherapy), but were off any treatment for at least 4 months before study entry. None of the patients showed any clinical or laboratory evidence of infection.

For the determination of sICAM-1 a commercially available sandwich enzyme immunoassay system (R&D Systems, Minneapolis, USA) was used in accordance with the manufacturer's instructions. In brief, the assay was performed in 96-well microtitre plates using triplicates of 100 µl serum, diluted 1:20. Plates were read on a Dynatech Immunoassay System (Dynatech Laboratories)

Table 1 Serum levels of sICAM-1 in patients with breast cancer

	n	Median (range)
Patients with early breast cancer		
T0	1	314 (–)
T1	18	292 (205–429)
T2	15	266 (198–483)
T3	1	298 (–)
T4	6	291 (272–310)
N^-	17	275 (198–483)
N^+	24	298 (204–391)
G1	2	264 (256–271)
G2	21	286 (215–483)
G3	18	298 (198–391)
ER^-	18	295 (199–429)
ER^+	23	279 (198–483)
PgR^-	21	314 (199–447)
PgR^+	20	271 (198–483)
Patients with metastatic breast cancer		
Lymph nodes: no	32	371 (213–1292)
yes	15	288 (153–686)
Bone: no	26	288 (153–1292)
yes	21	375 (285–705)
Liver: no	35	301 (153–705)
yes	12	385 (213–1292)
Lung: no	36	371 (153–1292)
yes	11	264 (213–403)
Brain: no	45	333 (153–1292)
yes	2	408 (403–413)

at 450 nm, with a correction wavelength of 620 nm. Concentrations of sICAM-1 were calculated from the mean OD of triplicates via correspondence to a standard curve generated by standard dilutions provided by the manufacturer. The limit of detection of the assay was <0.35 ng/ml. The intra- and inter-assay precision quoted for the sICAM-1 assay was between 3.3 and 4.8 and between 6.0 and 10.1% CV, respectively.

Flow cytofluorimetric analysis of HER-2/neu expression and ICAM-1 expression on breast cancer cell lines

HTB-25 (MDA-MB-175-VII), HTB-27 (MDA-MB-361), HTB-30 (SKBR-3), HTB 128 (MDA-MB-415), HTB-130 (MDA-MB-436), HTB-131 (MDA-MB-453) and HTB-132 (MDA-MB-486) cell lines were purchased from the American Type Culture Collection (Rockville, Md., USA). SKBR-3 cells were cultured in McCoy's modified medium (Gibco BRL, Paisley, Scotland) at 37 °C in a humidified atmosphere containing 5% CO_2; all other cell lines were cultured in Leibovitz's L-15 medium (PAA Laboratories, Linz, Austria) at 37 °C in a humidified air atmosphere. All media were supplemented with 10% fetal calf serum (FCS; Gibco BRL), 50 IU/ml penicillin and 50 µg/ml streptomycin (Gibco BRL). Cells were grown as monolayers in T75 flasks (Falcon, Lincoln Park, N.J., USA) by seeding 5×10^6 cells in 25 ml of appropriate medium.

To harvest cells, monolayers were briefly trypsinised (Viralex Trypsin/EDTA; PAA Laboratories, Linz, Austria), vigorously vortexed and washed (5 min at 400 *g*) twice in phosphate-buffered saline (PBS Dulbecco's; Gibco BRL, Paisley, Scotland). Subsequently, 10^6 cells/sample were washed in phenol-red-free Hanks' balanced salt solution (HBSS; Gibco BRL) containing 0.3% bovine serum albumin (BSA; Sigma, St. Louis, Mo., USA) and 0.1% sodium azide (Merck, Darmstadt, Germany) and incubated with 50 µl 20% AB-group serum (Biotest, Dreieich, Germany) in HBSS at room temperature for 30 min. Afterwards, cells were washed twice with HBSS, incubated with 50 µl of 1:20 diluted monoclonal mouse anti-Her-2/neu antibody (Zymed Laboratories, Calif., USA) for 30 min on ice, and isotype-matched mouse antibodies (Isotypic control IgG_1-PE; Immunotech, France) were used as controls in a dilution of 1:2.5. Afterwards, cells were washed in PBS and incubated with fluorescein isothiocyanate (FITC)-conjugated goat $F(ab')_2$ antibodies to mouse IgG (Immunotech, France) for 30 min on ice, washed twice and resuspended in 300 µl staining buffer, i.e. HBSS supplemented with 1 µg/ml 7-amino-actinomycin-D (7-AAD; Sigma, Germany), to allow exclusion of dead cells. Cytofluorometric analysis was performed using a FACScan (Becton-Dickinson, San Jose, Calif.) according to the manufacturer's instructions. Forward and side-scatter cell gating was performed to detect fluorescence only on intact living cells. Background fluorescence was assessed using murine IgG1 (20 µl per sample, see above). Results were calculated using the LYSYS II software. The percentage of positive cells and mean channel fluorescence intensity (MFI), as well as fluorescence histograms, were recorded for each sample. All experiments were done in triplicate.

Determination of cytotoxicity by ^{51}Cr release

HTB-132 or SKBR-3 cells were harvested and resuspended in 100 µl of appropriate medium supplemented with 10% heat-inactivated (56 °C for 30 min) FCS. Subsequently, 100 µl of sterile radioactive sodium chromate (50 µCi, Du Pont Pharma S.A., Belgium) were added, the cells incubated in a 37 °C atmosphere for 60 min, washed three times and chromated targets resuspended with 100 µl of complete medium containing 10% heat-inactivated FCS.

Peripheral blood mononuclear cells (PBMC) from patients and controls were separated by a buoyant density gradient on Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) from freshly drawn heparinised peripheral venous blood, diluted 1:1 with PBS (30 min at 1000 *g*), washed three times (10 min at 1000 *g*) in 0.9% saline and resuspended in medium supplemented with 10% heat-inactivated FCS. After the cell number of PBMC was determined, using Zapoglobin (Coulter Electronics Ltd., England) to exclude erythrocytes, PBMC and tumour cells (1.25×10^4 target cells in 100 µl) were co-incubated for 4 h at an effector/target ratio of 25:1 and appropriate medium was added to a total volume of 1 ml. All samples were run in triplicates. After the incubation reaction, cells were centrifuged (10 min at 1000 *g*) and 500 µl of supernatant medium from each triplicate sample were transferred to polystyrol vials. Radioactivity was measured by liquid scintillation counting for 1 min in a γ-counter (Auto-Gamma Cobra, Packard, Canberra). Maximum lysis of tumour cells was measured 4 h after adding 100 µl of sodium dodecyl sulphate (0.5% SDS; Sigma Chemical Co, Mo., USA) and 800 µl of distilled water to 100 µl of tumour cell suspension and was always $>85\%$ of incorporated counts. Spontaneous ^{51}Cr release of tumour cells was assessed 4 h after incubation of tumour cells without lymphocytic effectors and was always $<25\%$ of total incorporated counts and $<10\%$ of the maximum release, respectively. Experimental lysis obtained in counts per minute (cpm) was calculated as percentage specific release:

$$\textit{Specific lysis}\ (\%) = 100 \times \frac{cpm\ (\textit{experimental release}) - cpm\ (\textit{spontaneous release})}{cpm\ (\textit{maximum release}) - cpm\ (\textit{spontaneous release})}$$

The standard deviation of triplicate counts was $<10\%$ of the mean.

Addition of anti-HER-2/neu antibody and sICAM-1

The concentration of anti-Her-2/neu antibody [Trastuzumab (Herceptin); Roche, Austria] added at the beginning of the co-incubation period and the effector/target ratio where half-maximal cytotoxicity occurred were determined by four-parameter fit analysis and found at 0.001 µg/ml and 25:1, respectively, resulting in a $33.7\pm7.4\%$ lysis of SKBR-3 cells (data not shown). In addition, 250 ng/ml, 500 ng/ml or 2000 ng/ml recombinant human ICAM-1 (R&D Systems, Minneapolis, USA) were added at the initiation of

cultures in the presence or absence of anti-Her-2/neu antibody. Overall, we analysed the effects of these cytokines and anti-Her-2/neu antibody, respectively, upon unspecific and antibody-mediated cytotoxicity in 14 patients (median age: 59.8 years; range: 33.0–72.2 years) with breast cancer metastatic to visceral organs and 8 healthy controls matched according to age (median age 55.0 years, range 42.3–57.8 years) and menopausal status (pre-menopausal: 7 vs. 4, respectively). None of the patients had ever received antineoplastic treatment (chemotherapy, radiotherapy, endocrine therapy, immunotherapy), nor did patients or controls show any clinical or laboratory evidence of infection. The results represent the means of triplicate determinations. In our experiments, cautious trypsinisation did not affect unspecific cytotoxicity, ADCC and Her-2/neu expression, respectively.

Antigen presentation and $CD4^+$ T cell proliferation

The impact of sICAM-1 upon antigen presentation was assessed in 34 healthy female controls and 30 patients with breast cancer metastatic to visceral organs matched according to age and menopausal status. None of the persons had any clinical or laboratory evidence of infection, nor had any of the patients received antineoplastic treatment within the last 6 months, i.e. most patients were analysed at the time of primary diagnosis of metastatic disease. Heparinised blood was drawn in the morning and immediately analysed as follows. After separation of PBMC, non-adherent cells were removed by repeated washing and $CD4^+$ T cells were isolated by magnetic immunoselection using a CD4-positive isolation kit (Dynal, Germany) in accordance with the manufacturer's instructions (as described in [44]) and analysed by flow cytometry; cell purity was 90–95% $CD4^+$ cells. Triplicate cultures containing adherence-purified monocytes (1×10^4/well, 90–95% purity, as determined by non-specific esterase staining, data not shown) and CD 4^+ T-enriched cells (1×10^5/well) were set up in flat-bottomed 96-well microtitre plates (Costar, N.Y., USA) and co-cultured with tetanus toxoid (5 μg/ml; Calbiochem, USA) from the start of the stimulation assay. Since kinetic studies evaluating the role of sICAM-1 upon autoreactive T cell proliferation have demonstrated that sICAM-1 acts in the early phase of interaction between T cells and antigen-presenting cells [34], we added sICAM-1 (at concentrations of 0 ng/ml, 250 ng/ml, 500 ng/ml and 2000 ng/ml) at the beginning of the co-incubation assay. Cells were kept for 5 days at 37 °C in a 5% CO_2 humidified atmosphere in RPMI-1640 medium supplemented with 10% heat-inactivated FCS, 100 IU/ml penicillin and 100 μl streptomycin. Maximum T cell proliferation was assessed by co-incubation of purified T cells with phytohaemagglutinin (PHA, 15 μg/well; Murex Biotech, UK). [^{3}H]-Thymidine (Amersham International Life Science, UK), at a concentration of 0.5 μCi/well was added for the final 16 h. Subsequently, plates were frozen at –30 °C, rethawed and the cells harvested with a Harvester Micromate 196 (Packard, Groningen, Netherlands) onto glass fibre filters (Packard, Groningen, Netherlands). The incorporation of [^{3}H]-thymidine into DNA was measured using a Direct Beta Counter-Matrix 96 (Packard, Groningen, Netherlands).

Statistical analysis

Concentrations of sICAM-1 are described by median and range, results of experiments on unspecific cytotoxicity, ADCC and antigen presentation by mean and standard deviation, respectively. Correlation of serum levels of sICAM-1 and tumour markers, CEA and CA 15-3, was assessed using the Spearman correlation test. Differences in serum sICAM-1 levels and levels of tumour markers CEA and CA 15-3, respectively, between patient subgroups were analysed by Wilcoxon rank sum test. The effects of sICAM-1 upon unspecific cytotoxicity, ADCC and antigen presentation were analysed by a two-sided Student's *t*-test. A *P* value smaller or equal to 5% was considered statistically significant. SPSS statistical software system (SPSS Inc., Chicago, Ill., version 10.0) was used for all calculations.

Results

Correlation of sICAM-1 with clinical parameters

sICAM-1 serum levels in patients with breast cancer

As shown in Table 1, median serum levels of sICAM-1 were 279.1 ng/ml (range 198.2–483.1 ng/ml) in patients with prior early breast cancer. In contrast, patients with advanced breast cancer presented with significantly higher median sICAM-1 serum levels of 333.6 ng/ml (range 153.1–1292.1 ng/ml, $P = 0.005$).

Correlation of sICAM-1 levels with clinical parameters and tumour markers in early breast cancer

Serum concentrations of sICAM-1 in patients with prior early cancer did not correlate with tumour size, lymph node involvement, nuclear grading, and oestrogen receptor and progesterone receptor status (data not shown). In patients with prior early breast cancer elevated tumour markers CEA (>5 μg/l in non-smokers, >10 μg/l in smokers) and CA 15-3 (>30 kU/l), respectively, were observed in 3/41 (7.3%) and 9/41 (22%) of patients, respectively, whereas only 1 patient (2.4%) presented with both elevated CEA and CA 15-3.

Correlation of serum sICAM-1 levels with clinical parameters in patients with advanced breast cancer

Table 1 shows sICAM-1 levels in relation to various clinical parameters in patients with metastatic breast cancer. Neither oestrogen nor progesterone receptor status significantly influenced sICAM-1 serum levels. Similarly, no differences were found between patients with tumours differing in histopathological grading or the number (1,2, ≥3) of organs involved by metastatic disease. Serum levels of sICAM-1 in patients with liver (median 384.6 ng/ml, range 213.4–1292.1) and/or bone metastases (median 375.4 ng/ml, range 285.0–704.8) were significantly higher compared to those observed in patients with disease metastatic to other sites, such as lung and/or lymph nodes (all *P* values <0.03). In patients with metastatic disease 22/47 (46.8%) and 32/47 (68,1%) of patients presented with elevated serum levels of CEA and CA 15-3, respectively, whereas only 9/47 (19%) patients with metastatic disease had both, normal values for CEA and CA 15-3. When comparing patients with prior early breast cancer and patients with metastatic disease, respectively, tumour markers CEA and CA 15-3 were significantly higher in the latter group (all *P* values <0.001). Finally, there was a positive correlation of sICAM-1 serum levels with serum concentrations of tumour markers CEA (Spearman rho $= 0.393$, $P = 0.006$) and CA 15-3 (Spearman rho $= 0.783$, $P < 0.001$) only in patients with metastatic disease.

Biological implications of the presence of sICAM-1

Cytotoxicity

Expression of the Her-2/neu protein in breast cancer cell lines. Out of the panel of 7 analysed breast cancer cell lines, the percentage of Her-2/neu protein-positive cells and mean channel fluorescence intensity (MFI) were highest in SKBR-3 (HTB-30) cells (>99% positive cells, MFI 985.68) and lowest in HTB-132 cells (1.82% and 3.29, respectively), whereas all other cell lines exhibited a significant, but weaker degree of Her-2/neu protein over-expression (data not shown). Thus, in all subsequent experiments evaluating the role of sICAM-1 upon unspecific and anti-Her-2/neu antibody-mediated ADCC, HTB-132 and SKBR-3 cells were used.

Influence of sICAM-1 upon unspecific and anti-Her-2/neu antibody-mediated cytotoxicity. In order to evaluate the role of sICAM-1 upon unspecific and antibody-mediated cytotoxicity, we performed ^{51}Cr-release experiments analysing the cytolytic rate of both Her-2/neu protein-positive SKBR-3 and Her-2/neu protein-negative HTB-132 breast cancer cell lines in the presence or absence of anti-Her-2/neu antibodies and sICAM-1 at concentrations observed in patients with early and advanced breast cancer, respectively. To exclude potential immunological effects of sICAM-1 upon long-term incubation that would have been missed in our experimental setting, we analysed the effects of sICAM-1 upon the lytic activity of immunological effector cells derived from healthy controls and from patients with metastatic breast cancer (Table 2). No difference in unspecific cytotoxicity against Her-2/neu-positive SKBR-3 cells was observed between patients (8.0 ± 4.2% lysis) and healthy controls (9.0 ± 5.3% lysis; $P = 0.91$). The addition of anti-Her-2/neu antibody significantly enhanced cytotoxicity against SKBR-3 cells in both patients (34.8 ± 13.1% lysis) and controls (35.6 ± 9.3%) compared to the respective cytolysis rates observed in the absence of anti-Her-2/neu antibody (all P values < 0.0001). Again, no difference in cytolytic rates was found between patients and controls, respectively, suggesting that the lytic activity of immunological effector cells remains intact in patients with metastatic breast cancer despite the presence of elevated serum levels of sICAM-1. To corroborate this observation, we added sICAM-1 at concentrations observed in patients with early breast cancer (250 ng/ml), metastatic breast cancer (500 ng/ml) and up to the highest levels observed in our serum analysis (2000 ng/ml). No influence upon unspecific or antibody-mediated cytotoxicity was found in any of the cohorts analysed (all P values > 0.05). No significant enhancement of cytotoxicity against Her-2/neu-negative HTB-132 cells was observed in patients or controls when anti-Her-2/neu antibody was added. Cytolysis rates of HTB-132 cells were 2.5 ± 1.7% with vs. 2.5 ± 1.1% without anti-Her-2/neu antibody in patients and 1.9 ± 2.0% vs. 1.9 ± 1.5% in controls (all P values > 0.05). As in SKBR-3 cells, the addition of sICAM-1 at various concentrations did not alter cytolysis of HTB-132 cells in the presence or absence of anti-Her-2/neu antibody in patients or controls, respectively. No significant cytolysis was observed upon co-incubation of anti-Her-2/neu antibody and tumour cells only.

Impact of sICAM-1 upon antigen presentation

Maximal T cell proliferation induced by stimulation of T cells by PHA was similar between patients and controls (15.047 ± 1.651 vs. 11.753 ± 1.401 cpm, $P > 0.05$). In accordance with our previously published observations [44], a decreased T cell proliferation subsequent to co-incubation with tetanus toxoid-pulsed APC was observed in patients with advanced breast cancer (585 ± 1.278 cpm) compared to healthy controls (1.596 ± 3.281 cpm). Thymidine uptake (as expressed in cpm ± SD) after co-incubation of tetanus toxoid-pulsed antigen-presenting cells and $CD4^+$ T cells in the absence or presence of sICAM-1 at concentrations of 250 ng/ml and 2000 ng/ml, respectively, was 1.596 ± 3.281, 1333 ± 2471 and 1548 ± 3092 in healthy controls and 585 ± 1.278, 739 ± 1626 and 590 ± 904 in patients, respectively. As shown in Fig. 1, the addition of sICAM-1 at median concentrations observed in sera of patients with early breast cancer (250 ng/ml) and up to the highest serum levels observed in patients with advanced breast cancer (2000 ng/ml) did not influence APC-induced T cell

Table 2 ^{51}Cr-release analysing the influence of sICAM-1 (at concentrations observed in the sera of patients with metastatic breast cancer) upon unspecific and anti-HER-2/neu antibody-mediated cytotoxicity directed against Her-2/neu-positive SKBR-3 cells and Her-2/neu-negative HTB-132 cells in patients ($n = 14$) with metastatic breast cancer and matched healthy controls ($n = 8$). *T* Tumour cells, *PBMC* peripheral blood mononuclear cells, *sICAM-1* soluble intercellular adhesion molecule 1; *anti-Her-2/neu* trastuzumab

	SKBR-3 cells		HTB-132 cells	
	Controls (%)	Patients (%)	Controls (%)	Patients (%)
T + PBMC	9.0 ± 5.3	8.0 ± 4.2	1.9 ± 2.0	2.5 ± 1.1(%)
T + PBMC + sICAM-1	9.4 ± 4.8	7.5 ± 3.6	2.3 ± 2.2	2.1 ± 1.7(%)
T + PBMC + anti-HER-2/neu antibody	35.6 ± 9.3	34.8 ± 13.1	1.9 ± 1.5	2.5 ± 1.7(%)
T + PBMC + sICAM-1 + anti-HER-2/neu antibody	38.2 ± 10.8	34.5 ± 12.5	2.5 ± 2.2	2.3 ± 2.6

proliferation in both, patients and controls (all P values > 0.05).

Discussion

Our findings of elevated serum levels in patients with various stages of breast cancer corroborate results of previous studies [4, 19, 32], which found preoperative and postoperative sICAM-1 levels in sera and cytosols to be elevated in patients with early breast cancer, compared to both healthy controls and patients with benign breast disease.

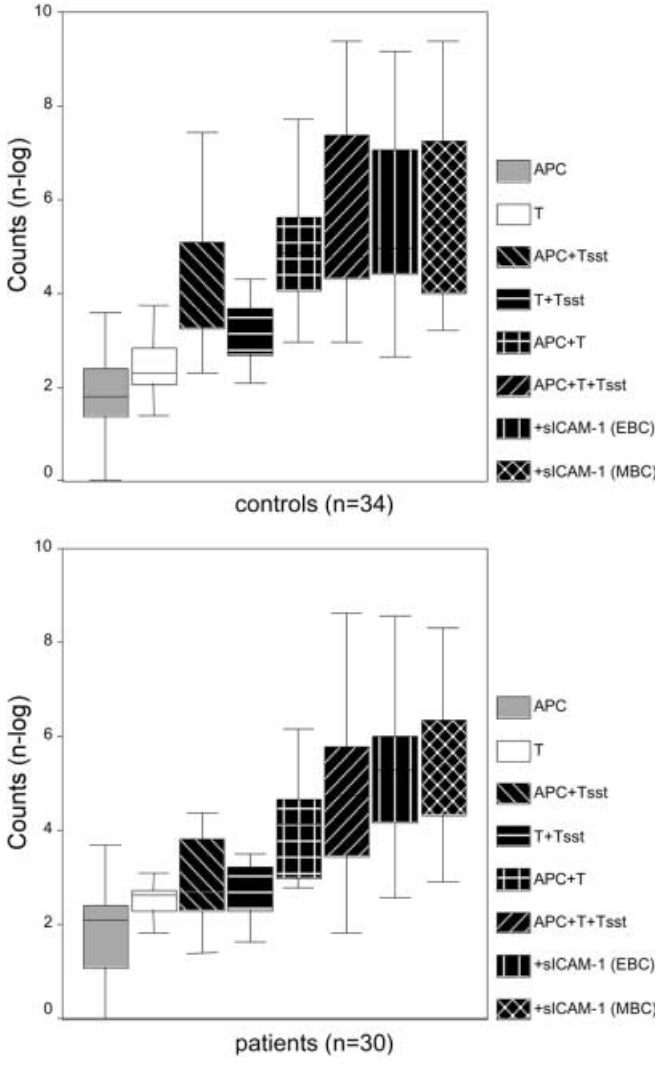

Fig. 1 Influence of sICAM-1 upon antigen presentation. A n-logarithmic presentation of ^{3}[H]-thymidine uptake after co-incubation of antigen-presenting cells (*APC*) and CD4-positive T cells (*T*). Experiments were performed in the presence or absence of tetanus toxoid (*Tsst*) and soluble intercellular adhesion molecule 1 (*sICAM-1*) at concentrations observed in the sera of patients with early (*EBC*) or metastatic (*MBC*) breast cancer in 34 healthy controls and 30 matched patients with metastatic breast cancer

Although analysed in a relatively small cohort and postoperatively, serum levels of sICAM-1 did not differ significantly between patients with not only various stages of early breast cancer, including lymph node involvement and tumour size, but also hormone receptor status and nuclear histopathological grading. In patients with metastatic breast cancer, serum levels of sICAM-1 were significantly higher compared to patients with early stages of disease. In contrast to other investigators, patients with metastatic disease were observed to present with a correlation between sICAM-1 serum levels and concentrations of the tumour markers CEA and CA 15-3. Similar to Zhang and Adachi [47], we found higher levels of sICAM-1 in patients with liver and/or bone metastases, but – in contrast to the latter study – no correlation of sICAM-1 levels with the number of metastatic sites. However, this may be due to the fact that we did not correlate serum levels of sICAM-1 with the total number and size of metastatic lesions, which probably represents tumour mass more appropriately compared to the number of organs involved by metastatic disease.

The origin of serum sICAM-1 and release mechanisms of ICAM-1 from the cellular surface remain unknown. Increased levels of sICAM-1 have been detected in cell culture supernatants and in sera of patients with ICAM-1-negative tumours and non-malignant diseases [14] indicating that, aside from malignant cells, also mononuclear cells [36], activated endothelia [14, 15, 22] and keratinocytes [9] could possibly constitute the source of increased serum levels of sICAM-1 observed in patients with breast cancer.

Concerning the biological role of sICAM-1, we have chosen the investigation of three role models, including unspecific cytotoxicity, ADCC and alloantigen-induced T cell proliferation, in which appropriate ICAM-1 expression has been shown to be decisively involved [11, 44]. While following this concept, it was speculated that the soluble form of ICAM-1 could interfere with ligands of ICAM-1 and thus inhibit regular interaction. Our results demonstrated that increased levels of sICAM-1 observed in the sera of patients with various stages of breast cancer were unlikely to either interfere with anti-Her-2/neu antibody-based adoptive immunotherapy or to further significantly contribute to defects in antigen presentation observed in patients with breast cancer. However, it also has to be borne in mind that distinct forms of sICAM-1, differing in molecular weight and functional characteristics, due to tissue-specific glycosylation or assembly to complexes, may be held responsible for the controversial functional role of sICAM-1 [25, 35, 39, 45].

In accordance with Cooley et al. [11] and our own results on defective antigen presentation in breast cancer [44], it could be speculated that ICAM-1/LFA-1 interaction might constitute only one out of several possible signals involved in tumour cell recognition and target cell binding, whereas additional signals might be required to determine whether a tumour cell is eliminated

by lysis. Thus, other counter-receptors of LFA-1, including ICAM-2 and ICAM-3, as well as several additional adhesion and costimulatory molecules, may have a significant impact upon the recognition and attack of malignant targets by cells of the immune system [16, 31]. Nevertheless, it is interesting to note that the solubilisation of ICAM-1 did not result in biological consequences in our experimental set-up or influenced mechanisms relevant for cytolysis or APC–T cell interaction. Thus, sICAM-1 seems to constitute a tumour marker signifying advanced disease of certain characteristics including liver and/or bone metastases, yet not exceeding the value of traditional tumour markers CEA and/or CA 15-3. We conclude, therefore, that the assessment of sICAM-1 does not result in a significant advantage for the judgement of either the clinical situation or the biological course of patients with early or advanced breast cancer.

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