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Original Paper

Serum Levels of Intercellular Adhesion Molecule 1 (ICAM-1) in Patients with Colorectal Cancer: Inhibitory Effect on Cytotoxicity

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A positive correlation between the level of ICAM-1 in serum and the stage of neoplastic processes has been demonstrated. We studied ICAM-1 serum concentration in 27 colorectal cancer patients and investigated the effect of this molecule on cellular aggregation and toxicity. ICAM-1 serum concentration in the group of patients was significantly higher ($P < 0.01$) than in normal controls and was related to tumour stage. Patient sera inhibited both the formation of cellular aggregates and the percentage of specific lysis, the effect being lost when the serum was depleted of ICAM-1. These results suggest that the release of soluble ICAM-1 may represent a mechanism of tumour escape. © **1998 Published by Elsevier Science Ltd. All rights reserved.**

Key words: ICAM1, S-Icam1, colorectal cancer, cytotoxicity inhibition
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INTRODUCTION

ICAM-1 is a membrane sialoglycoprotein with a molecular weight which ranges between 70 and 120 kDa [1]. This molecule is limited to the endothelium, keratinocytes, fibroblasts, macrophages and T and B lymphocytes, since its expression may be induced by cytokines, such as IL-1, IL-4, IL-6, IFN-α or TNF-α, which are released in inflammatory disorders, auto-immune diseases or malignancies [2–6].

The role of ICAM-1 in immune vigilance against tumour cells is still to be defined. There are many studies suggesting that the induction of ICAM-1 molecules in tumour cells leads to an increase in the cytotoxicity response [7–10] because there is an increase in antigen recognition, regardless of the participation of other macromolecules [11, 12]. ICAM-1 expression has been associated with a favourable prognosis in some tumours such as hypernephroma [13] and in non-small cell lung carcinoma [14]. In contrast, an increase in the expression of ICAM-1 has also been associated with the progression of certain tumours, such as melanoma or colorectal cancer. In these tumours, the increase of adhesion molecules may promote the adhesion of circulating tumour cells to the vascular bed, the invasion of the basement membrane and the growth of new tumour cell colonies in the metastatic site [15–17].

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Together with ICAM-1 tissue expression, the existence of soluble forms of this molecule has been demonstrated in the serum of normal subjects, patients with auto-immune diseases, transplant rejection and malignancies [1]. In some cases, the increase in ICAM-1 serum levels in various neoplastic processes [18–22] has been associated with an unfavourable prognosis. An inverse correlation between survival and ICAM-1 serum levels has been observed in melanoma [21]. Using the soluble form of ICAM-1, obtained from the growth medium of a melanoma cell line, Becker and associates demonstrated an inhibitory effect on *in vitro* cellular cytotoxicity [19, 23]. This fact could explain the relationship between ICAM-1 expression and tumoral progression, bearing in mind that some of the soluble forms of ICAM-1 would be derived from tumour tissue [24]. Since, in tumour cell lines, the culture supernatant may block cytotoxicity, the effect of serum ICAM-1 in cancer patients remains unresolved.

In the present study, we evaluated the presence of soluble ICAM-1 in the sera of 27 patients with colorectal cancer. We analysed the relationship between the serum levels of ICAM-1 and tumoral extension, and then, the capacity of sICAM-1 to inhibit the normal cytotoxic response.

PATIENTS AND METHODS

Sera and patients

Sera were obtained from 27 patients with cancer of the colon and stored in aliquots at −70°C. The staging of

patients was carried out following the TNM classification proposed by the UICC (International Union Against Cancer) in 1992. In order to clarified present data, stages I and II were grouped together. No patient was undergoing specific treatment at in the moment of serum extraction nor presented with another pathology. The characteristics of the patients are shown in Table 1. 10 clinically healthy blood donors of both sexes were used as controls. Serum and peripheral blood lymphocytes (PBL) were obtained from them to carry out functional trials of aggregation and cellular toxicity together with quantification of soluble ICAM-1.

Detection of circulating ICAM-1

Serum levels of ICAM-1 were measured by ELISA using a monoclonal anti-ICAM-1 antibody (British Biotechnology Products Ltd, U.K.). We used 96 microtitre wells trays, coated with murine anti-ICAM-1 antibody, to which the soluble form of ICAM-1 would bind. The secondary antibody, which was added later, was conjugated to a peroxidase enzyme.

The assay was carried out by diluting 25 µl of serum from each patient with 475 µl of diluent. In the same way, a sample which contained recombinant soluble ICAM-1 suspended in human serum was diluted to a 20:1 solution to act as a serum control. 100 µl of the samples were added to successive wells, together with 100 µl of conjugated anti-ICAM-1-HRP. They were kept at room temperature for 90 min, the contents of each well were aspirated and the wells were washed with 300 µl of buffer washing solution. 100 µl of substrate made from a tetrametyl benzidine solution was added to each well and incubated at room temperature for 30 min. 100 µl of acid solution, which stops the substrate reaction, was then added and the optic density was determined at 450 nm. Each experiment was carried out in duplicate.

Assay of cellular aggregation

The assay of cellular aggregation has been detailed by others [25]. Briefly, K562 cells were used as target cells. This cell line was obtained from an erythromyeloid tumour with surface expression of ICAM-1 and was cultivated in RPMI 1640 media complemented with fetal calf serum at 10% (Gibco, BRL Laboratories, Gaithersburg, Maryland, U.S.A.), 2 mN L-glutamine, 100 U/ml penicillin and 50 µg/ml gentamicin (all reagents provided by Flow Laboratories, Irvine, U.K.) in an atmosphere with 5% CO_2 and a temperature of 37°C.

For the effector cell, we used PBL isolated from the blood of a healthy donor. Mononuclear cells were purified in a Ficoll-Hypaque gradient and subsequently depleted of monocytes by plastic adherence. The K562 cell/effector cell ratio was 1/100. The K562 cells were incubated with 5% CO_2 and with 50 µl of control serum. PBL was added in the proportion mentioned and cellular aggregation was observed with a Zeiss phase contrast microscope after 120 min, calculating the number of aggregates formed. This number was calculated from the percentage of aggregates formed with respect to the total number of target cells per field. Each experiment was carried out in duplicate, the value expressed being the mean of both calculations.

Assay of cellular cytoxicity

First we labelled the K562 cells with radioactive sodium dichromate (1 mC/ml ICN Pharmaceuticals, California, U.S.A.) for 90 min. Then 5×10^3 marked cells were used in 50 µl to which we added 50×10^4 PBL in 100 µl and 50 µl of serum from patients. The toxicity assay was performed in triplicate in 96 U-round-bottomed-well microtitre plates (Costar, Cambridge, Massachusetts, U.S.A.). For the control of spontaneous and total isotope release, the target cells were resuspended in 150 µl of culture medium with 10% Triton to

Table 1. *Patients' characteristics*

No.	Age (years)	Sex (M/F)	Tumour location	Histopathological grade	Stage
1	59	M	Rectum	Moderately	I
2	55	M	Colon	Well	III
3	71	M	Colon	Moderately	II
4	62	F	Rectum	Moderately	II
5	78	F	Rectum	Well	I
6	73	M	Colon	Well	IV (liver)
7	70	M	Colon	Moderately	IV (liver)
8	58	M	Colon	Well	III
9	52	M	Colon	Moderately	II
10	73	F	Rectum	Moderately	II
11	68	F	Colon	Moderately	III
12	51	M	Rectum	Well	III
13	70	M	Rectum	Moderately	IV (liver)
14	70	M	Colon	Moderately	II
15	58	M	Rectum	Moderately	II
16	75	F	Colon	Moderately	II
17	69	M	Colon	Well	III
18	64	F	Colon	Well	IV (liver)
19	53	M	Colon	Poorly	IV (liver)
20	72	F	Rectum	Well	III
21	67	M	Colon	Moderately	IV (liver)
22	75	M	Colon	Poorly	IV (lung)
23	65	M	Colon	Well	IV (lung)
24	39	M	Rectum	Poorly	IV (liver)
25	58	F	Rectum	Well	II
26	49	F	Colon	Moderately	IV (lung)
27	48	F	Colon	Well	IV (liver)

lyse the cellular membrane. Controls with serum from healthy donors were also included. The plate was incubated for 4 h at 37°C in 5% CO_2 and centrifuged for 10 min at 1500 rpm after which 100 µl of supernatant were collected and put into a gamma counter (Ultragamma LKB, U.S.A.). Specific lysis was determined as:

$$\frac{\text{Problem release} - \text{spontaneous release}}{\text{Total release} - \text{spontaneous release}} \times 100$$

Assay of immunoabsorbance

A high absorbance plate (Costar Cambridge, U.S.A.) was incubated all night at 4°C with 100 µl of monoclonal anti-ICAM-1 antibodies (British BPL, Oxford, U.K.) The wells were washed three times with an isotonic saline solution, 10 µg/ml of albumin per well were added and the plate was incubated for 2 h at 37°C. The wells were washed three times, 100 µl of serum were added and the plates were incubated for 2 h at 37°C. The serum was recuperated and put into two aliquots, one to be measured and the other to be used in the inhibition assay.

Statistical methods

Comparison between groups was made using the variance analysis or Student's *t*-test.

RESULTS

Serum levels of ICAM-1 in patients with colorectal cancer

The presence of sICAM-1 was evaluated in 27 patients with colorectal cancer in different stages of the tumoral process and in 10 healthy subjects. The serum concentration of ICAM-1 was elevated in all patients. The median level of ICAM-1 in the patient group was 547.81 ng/ml (range 438.05–657.58 ng/ml), significantly higher ($P < 0.01$) than in the control group (mean 203.10 ng/ml, range 135.60–270.60 ng/ml). As shown in Figure 1, when we analysed the results according to stage, a progressive increase in the levels of serum ICAM-1 was observed, reaching a mean of 774.09 ng/ml (617.69–930.49 ng/ml) for those patients with diagnosed metastatic disease. There was no significant difference between the control group and stages I/II, although a significant difference was observed when we compared the control group and the group of patients in stage III and IV ($P < 0.01$).

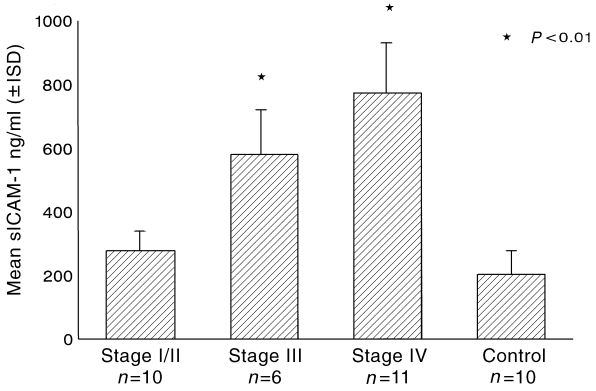

Figure 1. Serum levels of soluble ICAM-1 in patients with colorectal cancer. The levels of sICAM-1 were measured in the serum of 27 patients using a specific ELISA assay.

Inhibition of cellular aggregation by serum of patients with colorectal cancer

Since ICAM-1 is the coreceptor for the LFA-l molecule, we hypothesised that the soluble form of ICAM-1, present in the sera of colorectal cancer patients, may be a mechanism of immune escape for the tumour cells. To demonstrate this hypothesis, we performed a cytolytic assay between peripheral cytotoxic cells and the conventional target cells k562. We used sera from patients or healthy control subjects in order to analyse the ability of serum to affect K562 aggregation and subsequent lysis. A significantly lower mean percentage (40.92%; 36.78–45.08%) of aggregates was observed when patient sera was added to the assay. The inhibitory activity of the sera increased in proportion to the stage of the tumour, with a significantly lower percentage of aggregates in those assays performed with serum from patients with stage III and IV disease (Figure 2). In the assays of cellular cytotoxicity, serum from these patients produced a decrease in the percentage of specific lysis related to the stage (Figure 2).

Inhibitory capacity of ICAM-1 in cellular cytotoxicity

To demonstrate the involvement of soluble ICAM-1 in cell aggregate inhibition, sera from patients and healthy subjects were depleted with sICAM-1 and then used in assays of blockage of aggregation and cellular cytotoxicity. The immunoabsorption of sICAM-1 was confirmed (Table 2), and after depletion of sICAM-1, the sera from tumour patients failed to inhibit both cell aggregation and subsequent cell lysis.

DISCUSSION

Certain tumours are accompanied by an increase in the serum concentration of ICAM-1, which can be correlated to tumour progression and even constitute an unfavourable prognostic factor [20, 22]. We analysed the levels of sICAM-1 in 27 patients with colorectal cancer, supporting the existence of a correlation between tumour extension and ICAM-1

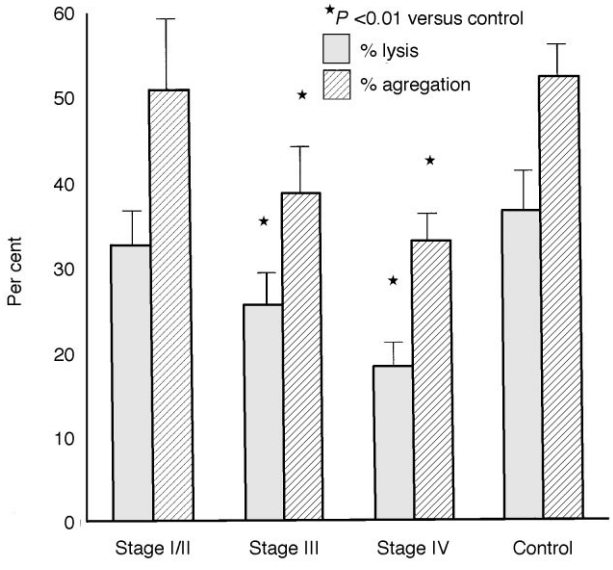

Figure 2. Ability of serum from colorectal cancer patients to inhibit cell aggregation and cytotoxicity. The percentage of cell lysis and cell aggregation between normal PBL and K562 target cells were determined before addition of serum from colorectal cancer patients.

Table 2. Percentage of aggregates and specific lysis obtained with serum from patients with colorectal cancer

Stage	Num.	% Aggregation		% Lysis		Mean serum level of ICAM-1 (ng/mg)
		Before*	After*	Before*	After*	
I/II	10	51.0 ± 6.7	49.3 ± 2.3	32.7 ± 3.8	36.2 ± 2.0	279.1 ± 77.8
III	6	38.6 ± 5.2	48.6 ± 1.8	25.5 ± 4.0	35.5 ± 4.9	580.8 ± 115.6
IV	11	33.0 ± 3.7	48.2 ± 2.4	18.2 ± 2.7	35.3 ± 1.6	774.1 ± 156.4
CONTROL	10	52.2 ± 3.7		36.4 ± 2.4		203 ± 67.5

*Before and after sICAM depletion. Mean ± S.D.

concentration in serum. Moreover, the functional blockage capacity of this molecule was demonstrated, since, if it was removed from the serum, the inhibitory effect was lost.

Several studies have shown an increase in the sICAM-1 levels in the serum of patients with breast, ovarian, gastrointestinal, melanoma and lymphoma cancer [18, 21, 22] and show that at least some of the soluble forms of ICAM-1 are derived from tumoral tissue [24]. The results of our series show an increase in the concentration of ICAM-1 in the serum of patients diagnosed with colorectal cancer and although the sample is limited, the concentration of ICAM-1 did relate to tumour stage. This has also been found in melanoma and in Hodgkin's disease [20, 22, 23] but others have found no relationship between sICAM-1 and stage [21].

Previous studies performed by our group confirmed the existence of an inhibitory effect on cellular toxicity by serum from patients diagnosed with metastatic carcinoma [26]. With respect to the factors involved in this resistance, these could range from factors released in every immune response such as IL-1, IL-6 or TNF, which have proved capable of inhibiting cytotoxic activity in given conditions [27], to cell surface macromolecules released into serum, such as the antigen CEA, a low-affinity receptor for IL-2 (rCD25), or ICAM-1, both capable of producing an inhibitory effect on the activity of NK and LAK cells [28–30].

The role of ICAM-1 in this process remains uncertain, since its expression at the cell surface level has been associated with an increase in cytotoxic response [9, 10], whilst in melanoma tissue expression of ICAM-1 is associated with an unfavourable diagnosis [15, 16]. Moreover, with regard to its soluble form, an inverse correlation between survival and serum levels of ICAM-1 has been shown in melanoma [21]. To explain this apparent contradiction, Becker and associates showed that sICAM-1 released into culture medium by melanoma cell lines led to inhibition of the cytotoxic effect of NK and TIL cells [19, 23]. For this reason, the ICAM-1 molecule could, in certain circumstances, cause inhibition of the cytotoxic effect. Subsequently, other groups have shown the immunosuppressor effect of soluble ICAM-1 [31].

In our series, serum from patients with colorectal cancer caused inhibition both in the formation of cellular aggregates and in the percentage of specific lysis, related to the concentration of soluble ICAM-1. The fact that the removal of these macromolecules from the serum was accompanied by loss of the inhibitory effect suggests that ICAM-1 can cause inhibition of cellular cytotoxicity, regardless of the existence of other factors.

Such findings lead us to consider the hypothesis that the release of soluble ICAM-1 could interfere in the recognition of the target cell by the effector cell, due to the blockage of the latter's coreceptors and could, therefore, be implicated in tumour escape mechanisms. In support of this hypothesis, it has recently been shown that the capacity of soluble ICAM-1 to bind to the LFA-1 molecule exists in relation to the degree of dimerisation [32], which also demonstrates that the quaternary structure of the circulating ICAM-1 can essentially affect its function.

In conclusion, we have shown that an increase in the serum concentration of ICAM-1 seems to be related to tumour stage in patients with colorectal cancer and that the removal of ICAM-1 molecules from the serum causes loss of the inhibitory effect which the serum of such patients has on cellular aggregation and cytotoxicity. For this reason, the ICAM-1 molecule could, in certain circumstances, constitute a tumour escape mechanism as it interferes with recognition of the tumour cell by blocking the effector cells' receptors. We are, at present, working on this hypothesis.

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