Clin Exp Immunol 1993; 92:412–418

Detection of a circulating form of vascular cell adhesion molecule-1: raised levels in rheumatoid arthritis and systemic lupus erythematosus

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(Accepted for publication 8 February 1993)

SUMMARY

We have developed a panel of MoAbs against four separate but overlapping epitopes on endothelial cell (EC) vascular cell adhesion molecule-1 (VCAM-1). Two of the MoAbs (1G11 and 1E5) inhibited T cell adhesion to tumour necrosis factor (TNF)-activated EC, whilst two MoAbs (1.4C3 and 6D9) did not. Using these MoAbs we have identified a circulating form of VCAM-1 (cVCAM-1) which has identical epitope distribution to the EC form, and which is able to support the adhesion of the human lymphoblastoid cell line Jurkat J6 by a VLA-4- and VCAM-1-dependent mechanism when immobilized from plasma. cVCAM-1 isolated by immunoaffinity and size-exclusion chromatographies was shown by SDS–PAGE to have an apparent mol. wt of 85–90 kD. Levels of cVCAM-1 were significantly raised ($P < 0{\cdot}001$) in rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) compared with normal individuals. It is possible that cVCAM-1 may be a useful plasma marker for the diagnosis and management of patients with inflammatory diseases. Furthermore, detection of elevated cVCAM-1 levels may act as a guide to the importance of VCAM-1-dependent cell adhesion in different pathological settings.

Keywords VCAM-1 adhesion lymphocyte rheumatoid arthritis systemic lupus erythematosus

INTRODUCTION

Vascular cell adhesion molecule-1 (VCAM-1) is a 105–110 kD single-chain glycoprotein belonging to the immunoglobulin supergene family [1]. VCAM-1 was first identified as a surface membrane molecule which acts as a ligand for very late antigen-4 (VLA-4), and thereby promotes the cell adhesion of lymphocytes, monocytes, eosinophils and basophils [2–4].

Although VCAM-1 was first characterized on vascular endothelial cells (EC), it is clear that a number of other cell types can also express this molecule. These include follicular dendritic cells, interdigitating reticulum cells, Kupffer cells, type B synovial lining cells and renal proximal tubule epithelial cells [5–9]. VCAM-1 expression on resting EC is minimal, but can be induced by stimulation with IL-1, tumour necrosis factor (TNF), lipopolysaccharide (LPS) or IL-4 [10,11]. The mechanisms leading to expression of VCAM-1 by cell types other than EC are currently not known.

The identification and tissue localization of cell surface molecules provide an indication of their involvement in inflammatory diseases such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). Many cell surface molecules can also be detected as soluble circulating forms, and their measurement has diagnostic potential in clinical settings where access to human tissue is limited. In the case of adhesion molecules, recent reports have demonstrated that intercellular adhesion molecule-1 (ICAM-1, CD54) and the hyaluronate receptor (CD44) can both be found circulating in the blood and in inflammatory fluids [12–15]. In this study we have used a panel of anti-VCAM-1 MoAbs to demonstrate the existence of a circulating, functionally active form of VCAM-1 (cVCAM-1).

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MATERIALS AND METHODS

Monoclonal antibodies

Anti-VCAM-1 MoAbs 1.4C3 and 1G11 were generated in our laboratory as previously described [10,16]. Two further MoAbs, 1E5 and 6D9, generated in our laboratory during the same fusion as MoAb 1G11, were characterized as reacting with VCAM-1 on the basis of an identical pattern of reactivity with TNF, IL-1 or IL-4-stimulated EC. Moreover, MoAbs 1E5, 6D9, 1.4C3 and 1G11 each specifically immunoprecipitated material of identical molecular mass from the eluate of a MoAb 1.4C3-agarose affinity column, and each reacts with mouse L-cells transfected with VCAM-1 cDNA. MoAbs were purified by affinity chromatography on goat anti-mouse immunoglobulin coupled to agarose. Antibodies were biotinylated by incubating

with biotinyl-ε-amino-caproic acid N-hydroxysuccinimide ester (Calbiochem Corporation, La Jolla, CA) for 4 h followed by dialysis against PBS.

MoAbs TS1/18 (anti-CD18, LFA-1β) (American Type Culture Collection (ATCC), Rockville, MD), TS1/22 (anti-CD11a, LFA-1α) (ATCC) and MoAb K20 (anti-CD29, VLA-β) (IgG2a; a kind gift from Professor Alain Bernard, Nice, France) were used as a 1:100 dilution of ascites. Affinity purified MoAb 163H (anti-CDw49d, VLA-α_4) was a kind gift from Dr Michael Gallatin (ICOS Corporation, Bothell, WA). Unless stated, all MoAbs used in the study are mouse IgG1.

Isolation and culture of EC

EC were obtained by collagenase (Type II; Sigma, Poole, UK) digestion of umbilical veins, using a modified method of Jaffe *et al.* [17], as previously described [10]. EC at confluence were removed from culture flasks with 0·125% trypsin-EDTA in Puck's saline A, 10 mM HEPES, and resuspended in EC growth medium at a concentration of 2×10^5 cells/ml. Aliquots (0·2 ml) were then cultured overnight or longer in flat-bottomed, gelatin-coated 96-well microtitre plates (Corning, Corning, NY), resulting in confluent monolayers as determined by phase contrast microscopy. Activation of EC by TNF was performed by incubation for 18 h with 10 ng/ml human recombinant TNF-α (a generous gift from Chiron Corporation, Emeryville, CA).

EC were fixed before using in ELISA by incubation in a solution of 2% paraformaldehyde, 100 mM L-lysine monohydrochloride, 2·14 mg/ml sodium meta-periodate for 10 min at 4°C [10]. Fixation was stopped by the aspiration of fixative and replacement with 100 mM glycine, 1·0% bovine serum albumin (BSA) (w/v) in Hank's balanced salt solution (HBSS; Flow Laboratories, Rickmansworth, UK) for at least 40 min to block reactive aldehyde groups.

Lysates of TNF-activated EC were prepared from EC monolayers that had been pretreated for 18 h with 10 ng/ml TNF-α. EC were incubated for 30 min with ice-cold lysing solution consisting of 4 mM EDTA, 50 mM Tris: HCl, pH 7·4 in 150 mM sodium chloride, with 0·5% Nonidet P-40, 1 μM pepstatin A, 2 μg/ml leupeptin and 1 mM PMSF (all from Sigma). After centrifugation to remove nuclear debris, lysates were stored at −70°C for use in assays.

Lymphocyte–EC adhesion assay

T lymphocytes were isolated from peripheral blood as previously described [16]. The human lymphoblastoid cell line Jurkat J6, derived from Jurkat and obtained from the European Collection of Animal Cell Cultures (Porton Down, Salisbury, UK), was cultured in 10% fetal calf serum (FCS) in RPMI 1640. T cells or J6 cells were radiolabelled by incubation for 90 min with ^{51}Cr, $Na_2{}^{51}CrO_4$, and washed before inclusion in adhesion assays. T cell adhesion to EC was measured using monolayers of human umbilical vein endothelial cells (HUVEC) cultured in 96-well plates, as described in detail previously [16]. Inhibition by anti-VCAM-1 MoAb was tested by preincubating EC monolayers with MoAb ascites (1:100 dilution) for 15 min before addition of the radiolabelled T cells to the EC monolayers. Following coculture for 60 min, non-adherent T cells were washed from the EC monolayers and remaining T cells lysed by the addition of an aqueous 1·0% solution of Nonidet P-40. The percentage of adherent lymphocytes was then calculated as follows:

$$\text{Per cent adhesion} = \frac{\text{ct/min in 0·1 ml lysate}}{\text{ct/min in 0·1 ml of original lymphocyte suspension}}$$

The percentage inhibition by MoAb of TNF-induced T–EC binding was calculated as follows:

$$\text{Per cent inhibition} = \left(1 - \frac{\delta \text{ in presence of MoAb}}{\delta \text{ in wells with control medium}}\right) \times 100$$

where δ = (% lymphocyte adhesion in cytokine-stimulated cultures) − (% lymphocyte adhesion in control unstimulated cultures).

ELISA

An ELISA was used to detect binding of MoAb to the surface of TNF-activated EC, as previously described in detail [10]. The ability of MoAbs to compete for binding to VCAM-1 was tested by preincubation of TNF-activated EC with saturating concentrations of an unlabelled MoAb for 30 min at room temperature, followed by addition into the incubation medium of a biotinylated MoAb. Following an incubation period of 1 h at room temperature, binding of the biotinylated MoAb was detected by incubation for 30 min at room temperature with a high mol. wt complex of streptavidin-biotin-peroxidase (Sera-lab, Crawley Down, UK). The ELISA was developed with 0·5 mg/ml *o*-phenylenediamine, 0·03% hydrogen peroxide (v/v) in a pH 5 citrate-phosphate buffer (substrate), and colour development stopped with 4 N sulphuric acid. The OD was measured at 491 nm using a Titertek ELISA plate reader (Flow).

A two-epitope 'sandwich' ELISA was designed to measure VCAM-1-like activity in solution. Plastic 96-well microtitre plates (Linbro, Flow) were precoated overnight with affinity-purified MoAb at 10 μg/ml in carbonate buffer (pH 9·6) and left to dry overnight at 37°C. Plates were then blocked with 1·0% BSA in PBS for 1 h. After washing with 0·25% Tween (v/v) in PBS, test samples were added for 2 h at room temperature. Ligand capture was then detected by addition of a biotinylated anti-VCAM-1 MoAb, followed sequentially by incubation with streptavidin-biotin-peroxidase and substrate. Colour development was then stopped and OD measured as above. All combinations of the four anti-VCAM-1 MoAbs were tested in this way (see Results), with the final assay comparing clinical samples utilizing MoAb 1E5 on the solid phase followed by biotinylated MoAb 1.4C3. Regressional analysis of 130 plasma samples in this assay indicated high interassay reproducibility (correlation coefficient between two assays, $r = 0·989$).

Clinical samples were tested following dilution 1:4 in PBS. Each sample was also incubated with control wells precoated with purified mouse immunoglobulin (10 μg/ml) (Sigma), and any non-specific reactivity subtracted from the test measurements. For quantification, a single plasma sample with a high level of cVCAM-1 was selected and titrated on each assay plate. Based upon comparison with this standard, results were expressed as U/ml, with the lower limit of detection being 0·5 U/ml. When compared with the immunoreactivity of a purified, recombinant soluble VCAM-1 (rsVCAM-1) construct (kindly provided by Dr Roy Lobb, Biogen, MA), 1 U of cVCAM-1 activity corresponded to 73 ng of rsVCAM-1.

Delipidation
Delipidation was performed by vigorous agitation of sample with three changes of 1,1,2-trichlorotrifluoroethane, followed by centrifugation at 10000 ***g***, 4°C for 15 min to remove precipitated material.

Ammonium sulphate precipitation
A plasma sample with a high level of cVCAM-1 was selected, treated with ammonium sulphate (BDH, Poole, UK) (final concentration 20% saturated ammonium sulphate solution (v/v) in the plasma), centrifuged at 10000 ***g***, 4°C for 15 min and the supernatant removed. The concentration of ammonium sulphate was then increased in a stepwise fashion to obtain supernatant concentrations of 30, 40, 50 and 60% saturated ammonium sulphate (v/v). The pellets obtained by centrifugation after each step were washed once in 60% saturated ammonium sulphate solution (v/v) in distilled, de-ionized water before being redissolved in PBS.

Immunoaffinity chromatography
MoAb 1.4C3 was covalently coupled to cyanogen bromide-activated Sepharose-4B (Pharmacia) by overnight, 4°C incubation of the Sepharose with purified MoAb in a citrate:phosphate buffer, pH 6·8, at a concentration of 5 mg protein/ml of gel, with gentle agitation. Unoccupied reactive groups on the activated Sepharose were blocked by the addition of ethanolamine (to a concentration of 50 mM; Sigma) and further incubation of the MoAb-gel slurry at 4°C, for 2 h, with gentle agitation. As determined by absorbance at 280 nm, gel-bound MoAb represented >98% of the MoAb in free solution originally added to the activated Sepharose. MoAb-labelled gel was then packed into a column barrel and pre-eluted before use by extensive washing with 50 mM triethylamine:NaOH, pH 12·5, followed by re-equilibration to neutral pH with PBS containing 0·05% sodium azide (BDH) (PBS/azide). This MoAb 1.4C3-Sepharose was stored at 4°C in PBS/azide.

A delipidated, 30–50% ammonium sulphate cut of plasma was precleared first by incubation with unlabelled Sepharose (Pharmacia) in suspension for 4 h at 4°C, and then by passage at room temperature over a precolumn packed with mouse IgG-agarose (Sigma). After passing the precleared material over the MoAb 1.4C3-Sepharose column, the column was washed extensively with 50 mM glycine:HCl, pH 10·0 in 150 mM saline and cVCAM-1 eluted using 50 mM triethylamine:HCl, pH 11·5. The eluate was immediately brought to a neutral pH by the addition of Tris (to a concentration of 50 mM; Sigma) and HCl.

Size-exclusion chromatography
Material obtained by anti-VCAM-1-immunoaffinity chromatography was centrifuged at 10000 ***g*** for 10 min and then further purified by passage over a Hydropore-5 size exclusion column (Rainin, MA). VCAM-1-like reactivity was measured in the column fractions by adsorption to the solid phase of 96-well ELISA plates (Linbro), followed by detection with biotinylated MoAb 1.4C3, streptavidin-biotin-peroxidase complex and substrate. Optical densities were then obtained following subtraction of background readings.

Silver-staining of SDS–PAGE resolved proteins
Samples derived by size exclusion chromatography were analysed by SDS–PAGE (7·5%T; 2·6%C) under reducing conditions using a modified method of Laemmli [18] and the proteins visualized using the method of Morrissey [19]. Briefly, following electrophoresis, the polyacrylamide gel was prefixed by soaking overnight in 50% (v/v) methanol, 10% (v/v) acetic acid. The gel was then fixed by soaking in 5% (v/v) methanol, 7% (v/v) acetic acid for 30 min, rinsed in distilled, de-ionized water (Millipore, Watford, UK) for 30 min, then soaked in 5 μg dithiothreitol (DTT: Stratagene, La Jolla, CA)/ml for 30 min. $AgNO_3$ (0·1% (w/v)) was substituted for the DTT (without rinsing the gel) and the gel left for a further 30 min. After this the gel was rinsed rapidly once with a small volume of distilled, de-ionized water and then twice with small volumes of 3% (w/v) Na_2CO_3, 0·02% (v/v) formaldehyde (developer). The gel was then incubated in 100 ml of developer at room temperature until sufficient staining of the resolved proteins had occurred. Stain deposition was stopped by the addition of 5 ml of 2·3 M citric acid (stop solution). After 10–15 min, the excess developer and stop solutions were discarded and the gel washed several times in distilled, de-ionized water. Distilled, de-ionized water was used to dissolve all reagents and gentle agitation of the gel in its various solutions was used throughout the procedure. Gels stained in this manner were photographed for permanent record.

Patients
Clinical samples were derived from patients attending Hammersmith Hospital and from a panel of 155 normal healthy controls (93 male, 62 female; age range 20–65, median 34 years). Authorization to study the clinical samples was obtained from the local ethical committee. The group of patients with RA consisted of 13 males and 67 females (age range 26–85, median 58 years) and conformed to the 1987 American Rheumatism Association (ARA) classification [20]. The group of patients with SLE consisted of 29 females and 1 male (age range 18–60, median 33·5 years) and conformed to the 1982 ARA classification [21]. Samples routinely consisted of plasma anticoagulated with 5% EDTA. However, levels of cVCAM-1 were equivalent in paired plasma and serum samples from 15 individuals with values covering the whole range of the assays. Sera collected from 54 healthy individuals volunteering blood for transfusion were therefore included as part of the normal control group.

Statistical analysis
The significance of differences in lymphocyte adhesion was tested using Student's *t*-test. Differences between clinical groups in levels of circulating adhesion molecules were tested using the Mann–Whitney *U*-test corrected for multiplicity of testing by the method of Bonferroni. Correlations between variables were analysed using Spearman rank coefficient.

RESULTS

Characterization of anti-VCAM-1 MoAbs
Four MoAbs that react with human VCAM-1 were tested for their ability to inhibit the binding of T cells to TNF-activated HUVEC. MoAbs 1G11 and 1E5 were found to inhibit TNF-induced adhesion by 77% and 66% respectively, and were no more inhibitory when used together (inhibition 74%) than when either was used alone. MoAbs 1.4C3 and 6D9 had no significant inhibitory effect on T–EC adhesion (Fig. 1).

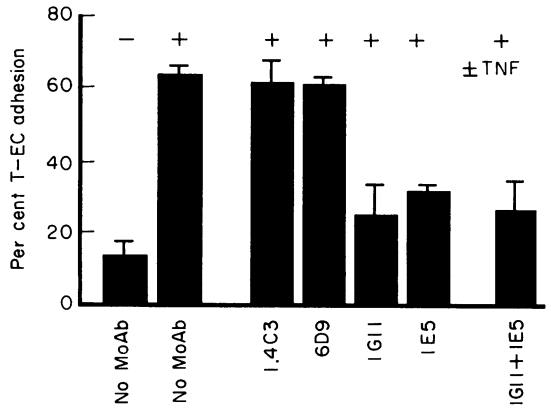

Fig. 1. Effect of four anti-VCAM-1 MoAbs on T cell adhesion to endothelial cell (EC) monolayers. EC were stimulated for 18 h with tumour necrosis factor (TNF) (10 ng/ml) (+) or medium control (−) before addition of ^{51}Cr-labelled T cells to the cultures. Results show the effects of anti-VCAM-1 MoAbs on TNF-induced T–EC adhesion compared with cultures incubated without MoAb. Similar results were obtained in two further experiments. Values are the mean ± s.d. of triplicates.

Cross-blocking experiments were next conducted using TNF-activated EC to determine the relationships between the epitopes on VCAM-1 recognized by these four MoAbs. As shown in Table 1, when binding of biotinylated MoAb to TNF-activated EC was studied in the presence of saturating concentrations of unlabelled monoclonals, complete competition was observed beween MoAbs 6D9 and 1.4C3 and between MoAbs 1.4C3 and 1G11. No reproducible competition was observed between any of the other combinations of the four MoAbs.

These observations suggested that it would be possible to use combinations of non-competing MoAbs to assay VCAM-1 in solution. Accordingly, microtitre wells were coated with each of the four anti-VCAM-1 MoAbs and then incubated with detergent lysates prepared from TNF-activated EC. As shown in Fig. 2a, captured antigen could be detected with a biotinylated second anti-VCAM-1, providing the biotinylated MoAb and the MoAb on the solid phase were not a combination previously determined to react with the same epitope.

Detection of a circulating form of VCAM-1

In order to test whether a circulating form of VCAM-1 was present in the blood of a patient with RA, a sample of plasma was assayed in parallel with lysate from TNF-activated EC. A similar pattern of MoAb reactivity was observed with the RA plasma as with the EC lysate (Fig. 2b). As predicted from the results shown in Table 1, it was possible to inhibit fully with MoAb 1G11 the binding of biotinylated MoAb 1.4C3 to antigen captured by solid-phase MoAb 1E5 from three different plasma samples or from TNF-activated EC lysate (data not shown). This immunoreactivity of plasma with anti-VCAM-1 MoAb was not affected by delipidation (data not shown), suggesting that it was not associated with circulating fragments of cell membranes.

To test whether the antigen captured from plasma by anti-VCAM-1 MoAb also had the functional qualities of VCAM-1, we used the human lymphoblastoid cell line Jurkat J6, which is known to bind VCAM-1 via cell-surface VLA-4 molecules [1,2]. Ninety-six-well microtitre plates were coated with the non-inhibitory anti-VCAM-1 MoAb 6D9, incubated for 16 h with RA plasma and then washed before addition of ^{51}Cr-labelled J6 cells. As can be seen in Fig. 3, wells incubated with RA plasma showed significantly enhanced adhesion (25·0 ± 5·0%) compared with wells incubated with tissue culture medium alone (4·1 ± 1·9%) ($P < 0·001$; Student's t-test). This enhanced adhesion was fully inhibited by MoAb directed against either the α or β chains of VLA-4, but not by MoAb directed against the α or β chains of LFA-1. Furthermore, the anti-VCAM-1 MoAb 1G11 was as inhibitory as anti-VLA-4 MoAb, supporting the conclusion that the J6 cells were binding VCAM-1 immobilized from plasma. MoAb 1G11 does not react with immobilized plasma fibronectin, another ligand for VLA-4 on leucocytes.

Effect of ammonium sulphate precipitation

As a preliminary to further purification of cVCAM-1, proteins in plasma were precipitated by stepwise addition of ammonium sulphate. cVCAM-1 activity was found to precipitate in the

Table 1*. Cross-blocking of anti-VCAM-1 MoAbs

		Unbiotinylated competing anti-VCAM-1 MoAb			
Biotinylated anti-VCAM-1	No antibody†	1.4C3	1G11	6D9	1E5
1.4C3	0·676 ± 0·676	0·045 ± 0·014	0·099 ± 0·022	0·080 ± 0·008	0·778 ± 0·165
1G11	0·594 ± 0·235	0·058 ± 0·005	0·057 ± 0·008	0·395 ± 0·045	0·514 ± 0·066
6D9	0·725 ± 0·012	0·069 ± 0·001	0·527 ± 0·060	0·045 ± 0·007	0·586 ± 0·121
1E5	0·690 ± 0·081	0·510 ± 0·105	0·508 ± 0·091	0·635 ± 0·144	0·192 ± 0·025

* Endothelial cells (EC) were stimulated with tumour necrosis factor (TNF) (10 ng/ml) for 18 h and fixed before measuring competition for VCAM-1 binding between the four anti-VCAM-1 MoAbs (see Materials and Methods).

† Apparent differences in the binding of biotinylated MoAb in the absence of competing MoAb were attributable to differences in the biotinylation rather than interaction with antigen. Binding of the biotinylated MoAb to unstimulated EC was 0·047 ± 0·005 for 1.4C3, 0·069 ± 0·020 for 1G11, 0·065 ± 0·011 for 6D9 and 0·161 ± 0·027 for 1E5. The results show data from an experiment using each of the four biotinylated MoAbs on a single batch of TNF-stimulated EC. Similar results were obtained in replicate experiments with biotinylated 1.4C3 (three other experiments), biotinylated 1G11 (three other experiments), biotinylated 1E5 (two other experiments) and biotinylated 6D9 (one other experiment).

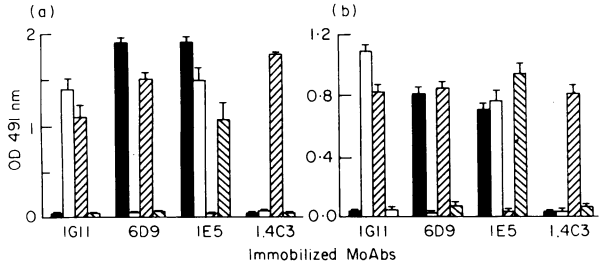

Fig. 2. Capture of antigen by anti-VCAM-1 MoAbs. Each of the four anti-VCAM-1 MoAbs were coated onto microtitre wells and used to capture antigen from (a) a lysate of tumour necrosis factor (TNF)-activated endothelial cells (EC) or (b) plasma from a patient with rheumatoid arthritis (RA). The ability of different biotinylated anti-VCAM-1 MoAbs to detect immobilized antigen was then assessed using streptavidin-biotinyl horseradish peroxidase (HRP) complex and substrate. Similar results were obtained in two further experiments. Values are the mean ± s.d. of triplicates. ■, Biotin 1G11; □, biotin 6D9; ▨, biotin 1E5; ▧, biotin 1.4C3.

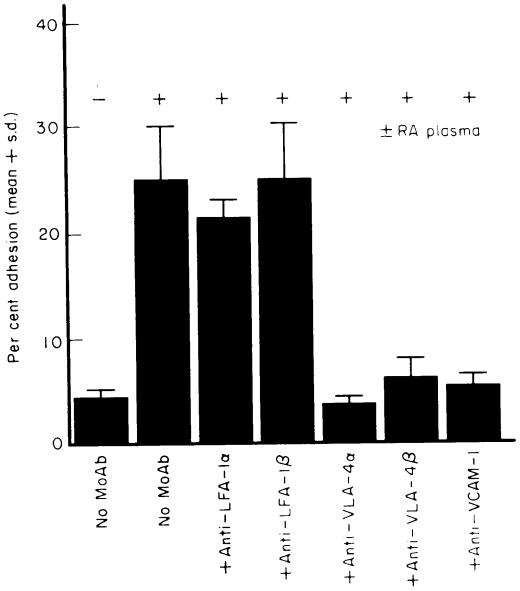

Fig. 3. Adhesion of J6 cells to cVCAM-1 captured by MoAb 6D9. Microtitre wells were coated with the non-inhibitory anti-VCAM-1 MoAb 6D9 and incubated with plasma from a patient with rheumatoid arthritis (RA). The increased adhesion in wells incubated with RA plasma was inhibited by MoAb against VLA-4 or VCAM-1 but not by MoAb against LFA-1. The experiment shown is representative of three similar experiments. Values are the mean ± s.d. of triplicates.

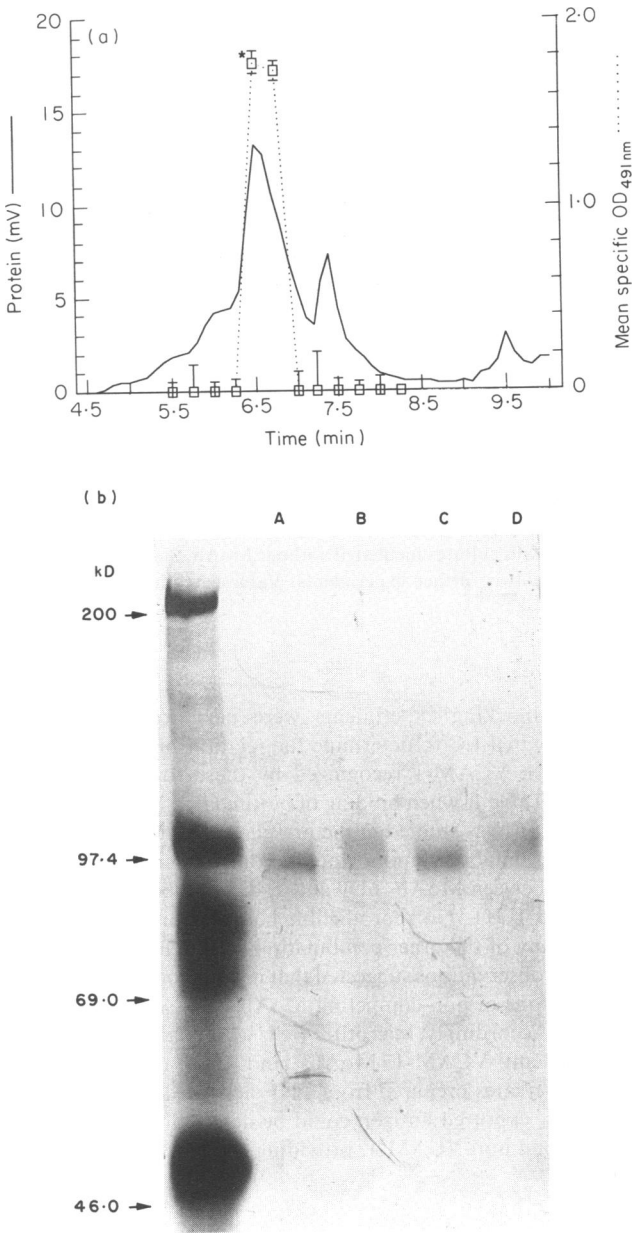

Fig. 4. Isolation of cVCAM-1 from plasma. (a) Following salt precipitation and immunoaffinity column chromatography over MoAb 1.4C3-agarose, cVCAM-1-containing material was passed over a size-exclusion chromatography column and analysed by ELISA (see Materials and Methods). (b) SDS PAGE (7·5%T; 2·6%C) followed by silver nitrate staining of the peak fraction of cVCAM-1 activity (* Fig. 4a) obtained from the size exclusion chromatography column (lanes A and C), and of rsVCAM-1 (lanes B and D).

30–40% and 40–50% cuts, as determined by capture ELISA (data not shown).

Size-exclusion chromatography and SDS–PAGE

Previous studies in this laboratory have shown that VCAM-1 immunoprecipitated from EC lysates has an apparent mol. wt of 105 kD by SDS–PAGE [16]. To address the question of whether the circulating form of VCAM-1 is of similar mass, cVCAM-1-containing material was extracted from plasma by ammonium sulphate precipitation followed by immunoaffinity chromatography over 1.4C3-agarose. The cVCAM-1 was then further purified by size-exclusion high performance liquid chromatography (HPLC) and fractions containing cVCAM-1 identified by capture ELISA (Fig. 4a). When analysed by SDS–PAGE and silver nitrate staining, cVCAM-1 demonstrated an apparent mol. wt of 85–90 kD (Fig. 4b, lanes A and C), approximately 5 kD lower than a rsVCAM-1 construct run in the same gel (lanes B and D).

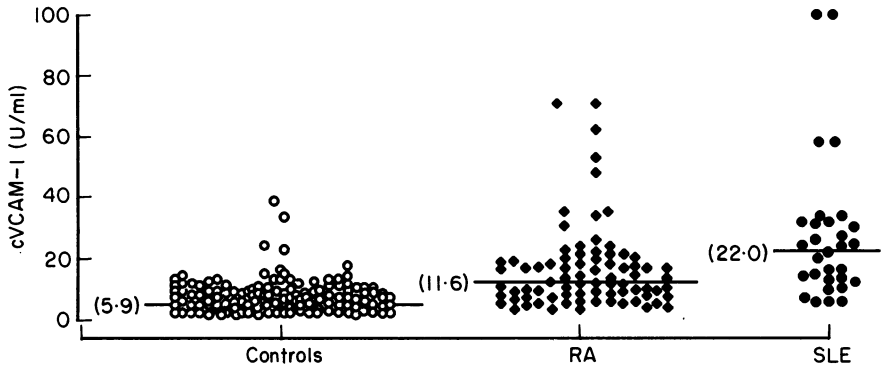

Fig. 5. Levels of cVCAM-1 in plasma. A sandwich ELISA employing MoAb 1E5 for capture and MoAb 1.4C3 for detection was used to measure levels of circulating VCAM-1 in the plasma of healthy volunteers (control group) and of patients with rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). Median cVCAM-1 activity for each group is represented by a horizontal line and a figure in parentheses.

Elevated blood levels of cVCAM-1 in RA and SLE

To determine whether the quantity of cVCAM-1 in the blood was altered in the context of inflammatory rheumatic diseases, we compared plasma from patients with RA or SLE with plasma from healthy volunteers. As shown in Fig. 5, levels of cVCAM-1 were significantly raised in both RA and SLE compared with the control group (RA $P < 0{\cdot}001$; SLE $P < 0{\cdot}001$; Mann–Whitney U-test). Examination of the control group showed no evidence for an effect of age on levels of cVCAM-1 ($r = 0{\cdot}06$, $P = 0{\cdot}466$; Spearman rank correlation).

DISCUSSION

We have used a panel of MoAbs to detect and quantify a circulating form of VCAM-1. The material detected by our capture ELISA demonstrates precisely the same pattern of overlapping epitopes as found with a detergent-solubilized VCAM-1 in a lysate of TNF-stimulated EC. Moreover, plasma containing the material was found to be capable of supporting lymphoid cell adhesion by a VLA-4/VCAM-1-dependent mechanism, demonstrating the capacity of the material to function as an adhesion molecule. It should, however, be realized that, although we have shown that immobilized cVCAM-1 can bind lymphoid cells, it remains to be determined whether the molecule has functional effects in the fluid phase, or whether the circulating molecule is of physiological significance.

At present it is unclear how cVCAM-1 is released by cells into extracellular fluid. There has been no report of an mRNA transcript lacking a transmembrane domain, and thus it seems likely that cVCAM-1 appears in plasma either as a result of proteolytic cleavage of membrane-bound VCAM-1, or as the result of shedding of the intact molecule. To address this question, we compared the apparent mol. wt of cVCAM-1 with that of rsVCAM-1, a construct formed by the insertion of a stop codon in the gene encoding VCAM-1 in place of the first amino acid residue of the transmembrane region (Leu-583) [1,22]. Our finding that the apparent mol. wt of cVCAM-1 was approximately 5 kD lower than that of rsVCAM-1 suggests that cVCAM-1 is formed by proteolytic cleavage of the extracellular region of membrane-bound VCAM-1 at a site near the membrane, with the small difference in apparent mol. wt between cVCAM-1 and rsVCAM-1 being explained predominantly by differential glycosylation between the native and recombinant forms (Dr Roy Lobb, personal communication). However, a direct comparison of the native circulating and membrane proteins is required to show the relationship between the two forms conclusively.

The detection of cVCAM-1 obviously does not indicate the anatomical or cellular source of the molecule. In a parallel study we have measured paired plasma and synovial fluid samples from RA patients with joint effusions, and have found significantly higher levels of cVCAM-1 in synovial fluid than in plasma [23], suggesting that one source of raised cVCAM-1 levels in RA is the synovium. Immunocytochemical staining of synovium has shown that VCAM-1 is particularly highly expressed by type B synovial lining cells which are found in the superficial synovial lining layer [24], and these cells may well be a major source of the cVCAM-1 in RA.

We have demonstrated for the first time the existence of a circulating form of VCAM-1. We are currently analysing further the relationship of levels of cVCAM-1 with levels of cICAM-1, and of levels of cVCAM-1 with other markers of disease activity such as erythrocyte sedimentation rate and C-reactive protein, to see whether measurement of this molecule in plasma and other extracellular fluids is useful for the laboratory assessment of patients with RA and SLE. In a wider clinical context, cVCAM-1 may also provide a guide as to the clinical settings in which to attempt the pharmacological blockade or manipulation of this adhesion molecule.

ACKNOWLEDGMENTS

We gratefully acknowledge the support of the Wellcome Trust.

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