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Cell-Adhesive Immunoglobulin M in Human Plasma†

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ABSTRACT: Human plasma contains a cell-adhesive protein that has a structure related to immunoglobulins. This protein was purified by affinity chromatography on an elastin–Sepharose column and by Mono Q anion-exchange chromatography. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis under non-reducing and reducing conditions revealed that this protein is a kind of immunoglobulin M (IgM). Antibodies against the μ chain and against the Fc region of IgM inhibited the adhesion of cells to this protein. Addition of the peptide GRGDS into media inhibited the adhesion, too. These results suggest that this protein is a special subset of IgM having a cell-binding sequence in the Fc region. We propose the name "cell-adhesive immunoglobulin M (CA-IgM)" for this protein. CA-IgM binds to α-elastin and laminin, suggesting that it may play a role in the interaction between cells and the extracellular matrix.

It has been shown that cellular adhesion on extracellular matrix proteins plays an important role in events such as embryonic development and cancer metastasis (Alberts et al., 1983). It has also been shown that blood plasma contains several cell-adhesive proteins such as fibronectin, chondronectin, and vitronectin (Yamada, 1983). Cells adhere to and spread on solid surfaces that have been coated with these proteins.

Recently, the relationship between extracellular matrix and adhesive proteins was elucidated. That is, adhesive proteins can interact with extracellular matrix components. For example, fibronectin, laminin, and vitronectin interact with collagens and glycosaminoglycans (Yamada, 1983). These interactions may play important roles in cell–cell or cell–extracellular matrix communications. However, no cell-adhesive

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protein has been known which binds to elastin, a major extracellular matrix protein.

In the previous study, we found a new cell-binding protein whose molecular weight is higher than 900 000 in human plasma (Ohori et al., 1986; Fukamizu et al., 1986). The protein seemed to contain subunits identical with or closely related to heavy chains of immunoglobulins, i.e., the μ chain of IgM, the γ chain of IgG, and the α chain of IgA. We tentatively called this protein "cell-binding immunoglobulin-like protein". In this study, we further purified this protein and investigated its structure, adhesion activity to the cells, and the binding activity to the extracellular matrix. The protein was found to be a kind of immunoglobulin M. Therefore, we now propose the name "cell-adhesive immunoglobulin M (CA-IgM)" for this protein.

Experimental Procedures

Materials. Human plasma was supplied by Toho University School of Medicine Hospital and the Blood Center of the Japanese Red Cross. Fibronectin was purified from fresh normal human plasma by gelatin affinity chromatography (Engvall & Ruoslahti, 1977). Laminin and peptides were kindly provided by Dr. K. Horiuchi, Hamamatsu University School of Medicine. Elastin from bovine neck ligament was obtained from Sigma (St. Louis, MO). Solubilized elastin (α-elastin) was prepared as described previously (Mecham & Lange, 1982). Other chemicals were of the purest grade available commercially.

Cells and Cell Culture. Rat embryo fibroblast 3Y1 and SV40-transformed W3Y were cultured in Eagle's minimal essential medium (MEM)[1] supplemented with 12% fetal calf serum (FCS). Mouse melanoma B16 were obtained from the Japanese Cancer Research Resources Bank and cultured in MEM containing 10% FCS. Human iliac vein endothelial cells (HIVE 24) were obtained from Dr. E. Levine, Wistar Institute, and cultured in M199 supplemented with 20% FCS, 1% endothelial cell growth factor (ECGF), and 0.1 mg/mL heparin (Thornton et al., 1983).

Preparation of Elastin–Sepharose. Elastin–Sepharose was prepared by coupling the solubilized elastin (α-elastin) with activated CH-Sepharose 4B according to the methods of Ohori et al. (1986).

Purification of CA-IgM. Human plasma (about 100 mL) mixed with diisopropyl fluorophosphate (final concentration 1 mM) was passed through a Sepharose 6B column (2.0 × 25 cm) and then applied to an elastin–Sepharose column (1.8 × 20 cm) equilibrated with 0.1 M NaCl–0.05 M Tris-HCl, pH 7.4 (TBS). After being washed with TBS, the column was eluted with TBS containing 4 M urea. The cell-adhesive activity peak was pooled, dialyzed against 0.16 M Na_2HPO_4–KH_2PO_4 (pH 6.5), and then subjected to Mono Q 10/10 anion-exchange chromatography. Elution was performed with 0.16 M Na_2HPO_4–KH_2PO_4 (pH 6.5) containing NaCl. The NaCl concentration was indicated in Figure 2. The peak fraction was rechromatographed on the Mono Q column. The final yield was 0.2–0.5 mg from 100 mL of plasma.

The concentration of protein was measured with the Bio-Rad protein assay kit (Bio-Rad Laboratories, Richmond, CA).

Electrophoresis. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was performed according to the method of Laemmli (1970) in 4.2% or 10% acrylamide gels. Molecular weight markers were from Sigma (St. Louis, MO). The gels were stained with the silver stain kit (Kanto, Tokyo, Japan).

Electrophoretic Transfer of Proteins to Nitrocellulose. After SDS–PAGE, proteins were transferred to the nitrocellulose filter (Toyo, Tokyo, Japan) according to the method of Towbin et al. (1979). Electrophoretic transfer was performed at 4 °C with 100 mA for 20 h.

Immunological Detection of Protein. The nitrocellulose filter was immersed in phosphate-buffered saline (PBS) for 1 h at room temperature, washed with PBS (pH 7.2) containing 0.05% Tween 20 (PBS–Tween) for 5 min three times, incubated in PBS containing 3% (w/v) bovine serum albumin (BSA) for 1 h at 37 °C, washed with PBS–Tween three times, and incubated in horseradish peroxidase conjugated (HRP) goat anti-human IgM (μ-chain specific; Cappel, West Chester, PA) for 1 h at 37 °C. After the nitrocellulose filter was washed with PBS–Tween three times, it was immersed in a solution of 0.025% 3,3′-diaminobenzidene–0.03% H_2O_2–PBS. The reaction was stopped after 10–20 min by washing with water.

Cell Attachment to Filter. This was performed essentially according to the method of Hayman et al. (1982). A filter with transferred protein was washed with PBS(+) three times, then soaked overnight in 0.5% (w/v) ovalbumin to prevent nonspecific cell attachment to uncoated filter paper, and washed again with PBS(+) three times. A cell suspension in MEM (2×10^5 cells/mL, 10 mL) of 3Y1, which had been prepared with trypsin, was added to the filter and incubated for 2 h at 37 °C. The filter was washed with PBS(+), fixed with 0.1% glutaraldehyde, stained for 1–2 min with 0.1% amido black (Sigma Chemical Co., St. Louis, MO), 45% methanol, 10% glacial acetic acid, and 45% deionized water, and destained in 90% methanol, 2% acetic acid, and 8% water.

Proteolytic Digestion. Papain digestion was performed under conditions similar to those of Onoue et al. (1966). CA-IgM or IgM was dialyzed against PBS(−) and digested with 1/100 weight of papain (Sigma Chemical Co., St. Louis, MO) for 16 h at 37 °C in the presence of 0.01 M cysteine and 0.002 M ethylenediaminetetraacetic acid (EDTA). For tryptic digestion (Miller & Metzger, 1966), CA-IgM or IgM was dialyzed against 0.2 M Tris-HCl, pH 8.0, and incubated with 2/100 weight of trypsin (Sigma Chemical Co., St. Louis, MO) for 16 h at 37 °C in the presence of 0.01 M $CaCl_2$ and 0.002 M EDTA. After incubation, both digests were electrophoresed in SDS–15% polyacrylamide gel.

Adhesion Assay. The general procedure of this experiment was based on the method of Rauvala et al. (1981). CA-IgM, fibronectin, or ovalbumin was incubated at a protein concentration of 3–50 μg/mL in 50 μL of PBS(+) at 37 °C for 2 h in a flat-bottomed polystyrene microtiter plate (Nunc, Denmark). The wells were washed three times with 250 μL of PBS(+). After being coated with the adhesion-mediating proteins, the wells were saturated with 250 μL of 0.5% ovalbumin in PBS(+) at 37 °C for 1 h. Then the wells were washed again three times with 250 μL of PBS(+). For adhesion assay, cells were suspended in culture medium without FCS and other supplements, except in the case of HIVE 24. When HIVE 24 was used, 2 mg/mL BSA was added to the medium.

Adhesion assay was started by adding 1×10^6 cells in 100 μL into flat-bottomed microtiter wells. In the case of HIVE

[1] Abbreviations: Tris, tris(hydroxymethyl)aminomethane; TBS, Tris-buffered saline; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; MEM, Eagle's minimal essential medium; FCS, fetal calf serum; PBS, phosphate-buffered saline; PBS(+), Dulbecco's phosphate-buffered saline with Ca^{2+} and Mg^{2+}; PBS(−), Dulbecco's phosphate-buffered saline without Ca^{2+} and Mg^{2+}; HRP, horseradish peroxidase conjugated; ELISA, enzyme-linked immunosorbent assay.

24, 3×10^4 cells in 100 μL were added. After incubation for 2 h at 37 °C in 5% CO_2, the medium including nonattached cells was washed off by rinsing the wells three times with 250 μL of PBS(+). Then the attached cells were fixed with 0.1% glutaraldehyde in PBS(+) and stained with 0.1% hematoxylin and 1% eosin. To quantitate the extent of cell adhesion, the turbidity was measured at 492 nm with an EIA Reader, Model 2550 (Bio-Rad, Richmond, CA). The level of cells attaching to 30 μg/mL fibronectin was designated as 100%.

ELISA. Immunological assay of CA-IgM was performed by using enzyme-linked immunosorbent assay (ELISA) (Engvall, 1980) in polyethyrene microtiter plates Cobind (Micro Membrane, Inc., Newark, NJ).

For measurement of binding affinity of CA-IgM to various extracellular matrix components, the wells were coated by incubation at 37 °C for 1 h with 50-μL solutions containing 100 μg/mL extracellular matrix component. The wells were washed with PBS containing 0.1% (w/v) gelatin, successively, and then the plates were incubated with 1% (w/v) ovalbumin for 1 h to inhibit nonspecific binding. The plates were then incubated with CA-IgM or authentic IgM (Green Cross, Osaka, Japan) as control. After being washed in the same manner, they were incubated with HRP anti-μ-chain goat IgG (Cappel, West Chester, PA), diluted 1:100 in PBS containing 1% ovalbumin at 37 °C for 1 h. The antibody binding was detected by using Peroxidase Substrates System (Kirkegaard & Perry Laboratories Inc., Gaithersburg, MD). The colored products were measured spectrophotometrically at 405 nm after 10-min incubation.

For the detection of the binding sites of CA-IgM to elastin, first the wells were coated by incubation for 1 h at 37 °C with 50 μL of CA-IgM solution (50 μg/mL), washed with PBS containing 100 μg/mL gelatin, and then saturated with 100 μg/mL gelatin. Second, they were incubated for 1 h with 50 μL of goat antibodies against the μ chain (Tago, Burlingame, CA), Fc fragment (Nordic Immunological Laboratories, Tilburg, The Netherlands), κ chain, or λ chain (Tago, Burlingame, CA) and washed again. Third, the wells were incubated with 50 μL of α-elastin (100 μg/mL) for 1 h, washed as described above, and incubated with rabbit antibovine α-elastin antibody (Elastin Products Co. Inc, Pacific, MO) for 1 h. Finally, the binding of the anti-elastin antibody was detected by using HRP goat anti-rabbit IgG (Cappel, West Chester, PA).

Inhibition Studies with Antibodies or Peptides on Cell Adhesion of CA-IgM. After being coated with CA-IgM, the wells were saturated with ovalbumin and then incubated with 50 μL of anti μ chain, Fc fragment, κ chain, or λ chain at 37 °C for 1 h. Then the wells were washed three times with 250 μL of PBS(+), and the adhesion assay was carried out as described previously.

In order to test the effects of peptides on the cell-adhesive activity, the wells were coated with CA-IgM and saturated with ovalbumin. The cell suspension with peptides was added into the wells at the concentration described in the legend to Figure 10.

Results

Purification of CA-IgM. The potent cell-adhesive activity was found in the eluate from the elastin–Sepharose 4B column using 4 M urea (Figure 1). The peak was pooled and subjected to Mono Q anion-exchange chromatography. As shown in Figure 2, several 280-nm absorbing peaks and two cell-adhesive activity peaks appeared. The major active peak (marked with the bar in Figure 2) was further purified by rechromatography on the Mono Q column (not shown).

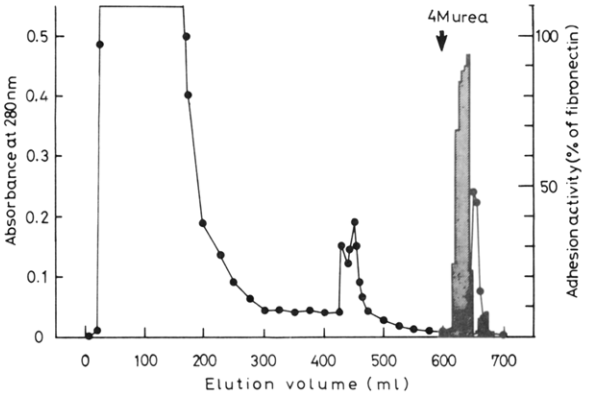

FIGURE 1: Affinity chromatography on an elastin–Sepharose column. A total of 100 mL of human plasma was applied to the column and eluted with 4 M urea (indicated by the arrow). Details of the procedure are described in the text. (●) Absorbance at 280 nm; (hatched area) adhesion activity.

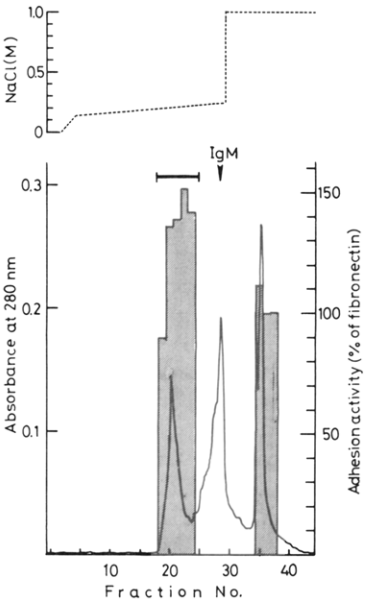

FIGURE 2: Elution pattern of CA-IgM from a Mono Q column. The cell-adhesive activity peak in Figure 1 was applied to the Mono Q column. After the column was washed with 50 mL of 0.16 M Na_2HPO_4–KH_2PO_4 (pH 6.5), elution was started; 1.5-mL fractions were collected. The elution position of commercially available IgM is shown by the arrow. (Solid line) Absorbance at 280 nm; (hatched area) adhesion activity.

SDS–PAGE showed that this protein was homogeneous (Figure 3A). The previous study indicated that the molecular weight of the cell-binding, immunoglobulin-like protein is larger than IgM. However, we found that commercially available IgM specimens were quite heterogenous with respect to SDS–PAGE mobility. The mobility of the purified sample on SDS–PAGE was found to be within the range of that of IgM and coinciding with that of IgM from normal human serum (Green Cross, Osaka, Japan). Immunological detection on nitrocellulose indicated that this protein reacts with anti-IgM (data not shown).

Cell attachment assay on nitrocellulose confirmed that this protein possesses cell-adhesive activity (Figure 3B). However, commercially available IgM did not show the cell-adhesive activity and was eluted from the Mono Q column at the position shown in Figure 2. Therefore, the cell-adhesive protein was different from the bulk of IgM.

The molecules responsible for the second peak of cell-adhesive activity in Figure 2 have not been characterized. SDS–PAGE analysis indicated that this fraction contained

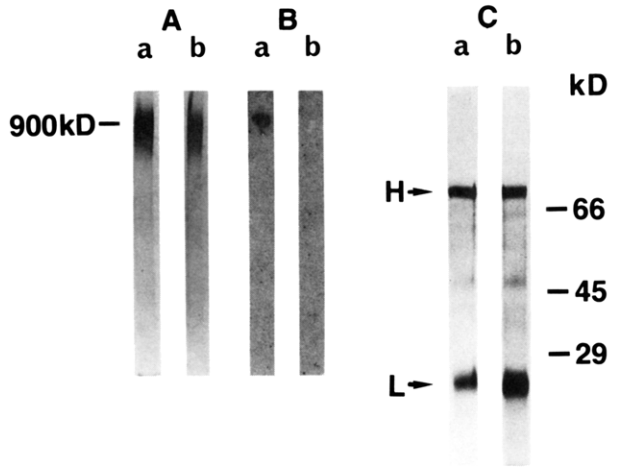

FIGURE 3: SDS–PAGE pattern. Details of the procedure are described in the text. CA-IgM (lane a) was compared with IgM (lane b). (A) Electrophoresed in the absence of 2-mercaptoethanol and stained by silver stain. (B) Electrophoresed in the absence of 2-mercaptoethanol and transferred to nitrocellulose. The cell attachment assay was performed with 3Y1 cells. Attached cells were stained with amido black. (C) Electrophoresed in the presence of 10 mM 2-mercaptoethanol and stained by silver stain. Arrows indicate the heavy (H) and light (L) chains.

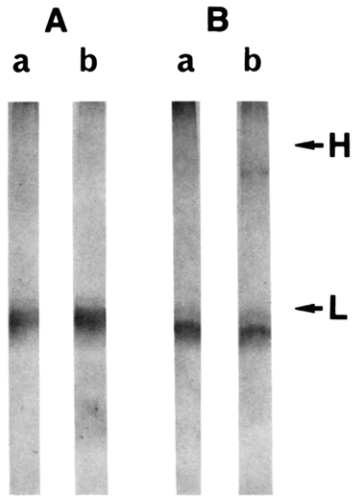

FIGURE 4: Peptide mapping by digestion with papain (A) and trypsin (B). CA-IgM (a) or IgM (b) was digested with the enzymes and electrophoresed in SDS–15% polyacrylamide gel. Arrows indicate the intact heavy (H) and light (L) chains of IgM. Details of the procedure are described in the text.

several proteins including IgM.

Structure of CA-IgM. SDS–PAGE under reducing conditions indicated that the protein was a disulfide-linked heteromultimer, consisting of one kind of heavy chain and one kind of light chain. The heavy chain was identical with that of IgM (Figure 3C). We have previously reported that this protein might have γ- and α-like chains of immunoglobulin in addition to the μ-like chain (Ohori et al., 1986). However, it seems that the previous results came from incomplete purification. The presence of the IgM-like structure in this protein was confirmed by peptide mapping after limited hydrolysis with trypsin and papain (Figure 4). These results suggest that this adhesive protein in human plasma is a special subset of IgM. We propose the name cell-adhesive IgM (CA-IgM) for this protein.

Interaction between CA-IgM and Extracellular Matrix Proteins. As shown in Figure 5, we studied the interaction of CA-IgM with various proteins. CA-IgM was found to have affinity to α-elastin and laminin. However, this protein could not bind to fibronectin or collagen. The result suggests that CA-IgM may play an important role in vivo, interacting with some extracellular matrix proteins such as elastin or laminin. Ordinary IgM did not bind to these matrix proteins.

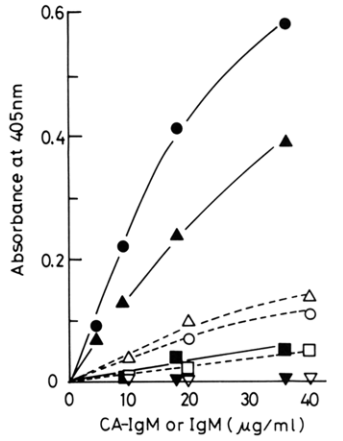

FIGURE 5: Interaction between CA-IgM and extracellular matrix proteins. Wells were coated with α-elastin, laminin, type II collagen, or fibronectin and then mixed with CA-IgM or IgM. Bound CA-IgM or IgM was detected with HRP goat anti-human IgM. Symbols: (●) α-elastin + CA-IgM, (○) α-elastin + IgM, (▲) laminin + CA-IgM, (△) laminin + IgM, (▼) type II collagen + CA-IgM, (▽) type II collagen + IgM, (■) fibronectin + CA-IgM, and (□) fibronectin + IgM.

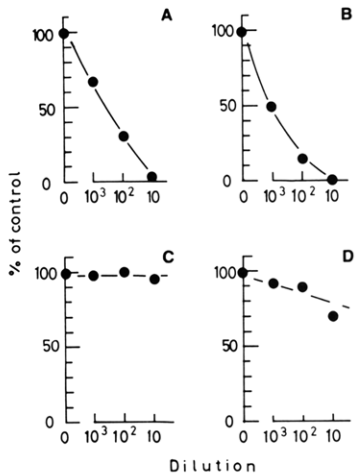

FIGURE 6: Effect of anti-IgM antibodies on CA-IgM binding to elastin. Wells were coated with CA-IgM and then treated with anti μ chain (A), anti Fc (B), anti κ chain (C), or anti λ chain (D). After washing, binding of α-elastin to CA-IgM was tested. Details of the procedures are described in the text.

The Fc region of the μ chain in CA-IgM seems to be involved in the binding to elastin, because the specific antibodies to the μ chain and Fc fragment inhibited the binding of CA-IgM to α-elastin (Figure 6).

Cell-Adhesive Activity of CA-IgM. The cell adhesion on the CA-IgM-coated well is shown in Figure 7. 3Y1 cells attached to and spread markedly on the CA-IgM-coated wells as on the fibronectin-coated wells.

The effect of protein concentration on the cell adhesion and time course of the adhesion were studied (Figure 8). Over 30 μg/mL, almost all cells were attached and spreading. In the time course, the adhesion was observed at 2 h.

Table I shows the results of the cell adhesion assay with different kinds of cells. It is of interest that normal cells, 3Y1, spread on the surfaces coated with CA-IgM, but transformed cells, W3Y, attached poorly to the same surfaces. However, B16 melanoma, which has very strong invasive activity, attached to the CA-IgM well.

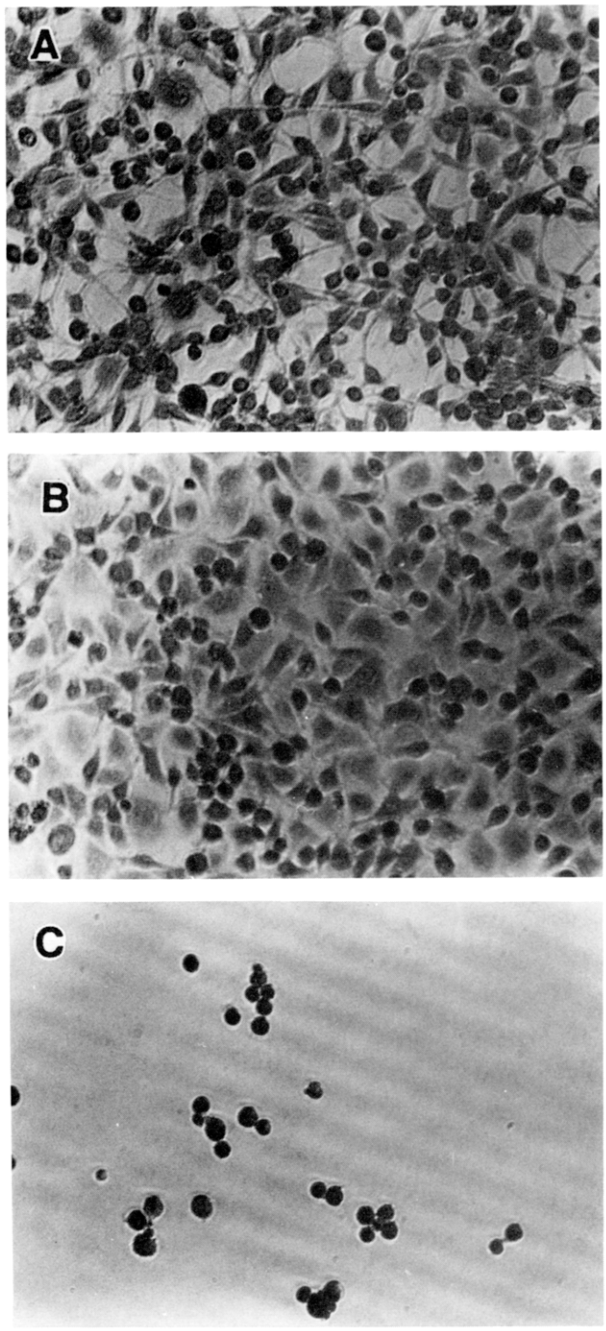

FIGURE 7: Adhesion of 3Y1 cells to surfaces coated with CA-IgM (A), fibronectin (B), or ovalbumin (C). The protein concentration used to coat the surfaces was 30 μg/mL.

Table I: Specificity of Cell Adhesion

cell type	adhesive surfaces			
	ovalbumin (500)[a]	fibronectin (50)	IgM (50)	CA-IgM (50)
fibroblast (3Y1)	–[b]	+++	–	+++
vein endothelial cell (HIVE 24)	–	+++	–	+++
transformed fibroblast (W3Y)	–	++	–	+
melanoma (B16)	–	+++	–	+++

[a] The protein concentration used to coat the surfaces (μg/mL). [b] +++, 75–100% saturation; ++, 50–75% saturation; +, 25–50% saturation; –, below 25% saturation.

Cell-Binding Sites in CA-IgM. The treatment of CA-IgM with anti μ chain or anti Fc fragment antibodies inhibited the cell-adhesive activity. This inhibitory effect could not be observed with anti κ or anti λ chain antibody (Figure 9).

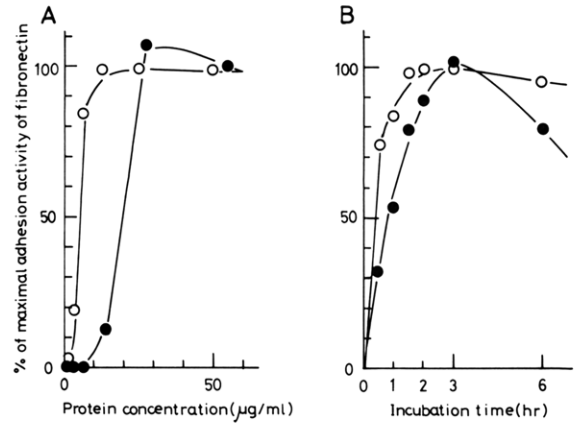

FIGURE 8: Attachment of 3Y1 cells to CA-IgM or fibronectin. (A) Effect of protein concentration. (B) Time course. CA-IgM (●) and fibronectin (○) were coated at 30 μg/mL. Protein coating and cell adhesion assay were performed as described in the text.

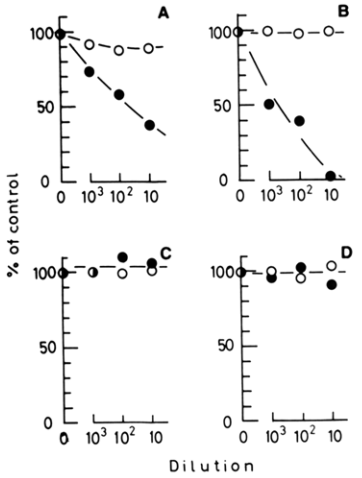

FIGURE 9: Inhibition of cell adhesion to CA-IgM or fibronectin by anti-human IgM antibodies. Wells were coated with CA-IgM (●) or fibronectin (○) and then incubated with the solution of anti μ chain (A), anti Fc (B), anti κ chain (C), or anti λ chain (D) for 1 h. The adhesion assay was performed with 3Y1 cells.

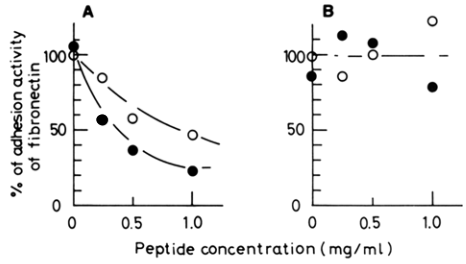

FIGURE 10: Effect of GRGDS (A) and GRGES (B) on cell-adhesive activity. The wells were coated with CA-IgM (●) or fibronectin (○). The adhesion assay was performed with 3Y1 cells. Details of the procedures are described in the text.

It has been shown that various cell-adhesive proteins including fibronectin, vitronectin, and type I collagen contain a common sequence, Arg-Gly-Asp (RGD), and small peptides containing the RGD sequence prevented the attachment of cells to these proteins (Pierschbacher & Ruoslahti, 1984; Hayman et al., 1985; Dedhar et al., 1987). We found that Gly-Arg-Gly-Asp-Ser (GRGDS) prevented the attachment of 3Y1 cells to CA-IgM, but Gly-Arg-Gly-Glu-Ser (GRGES) did not prevent the attachment (Figure 10). These results suggest that CA-IgM contains the RGD sequence in the Fc region of the μ chain, which serves as the cell-binding site.

Adhesive Activity of CA-IgM Interacted with α-Elastin. When the wells were first coated with α-elastin (100 μg/mL)

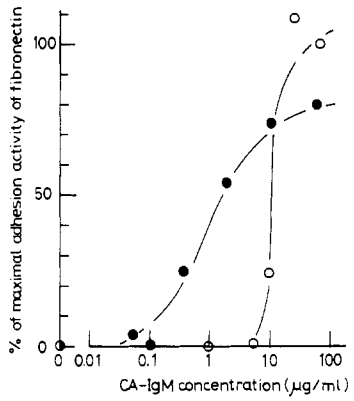

FIGURE 11: Effect of α-elastin on CA-IgM cell-adhesive activity. The wells were coated with CA-IgM only (○) or first coated with α-elastin and then coated with CA-IgM (●). The adhesion assay was performed with 3Y1 cells.

and then coated with CA-IgM (50 μg/mL), 3Y1 cells attached and spread very well (Figure 11). Particularly, at the low concentration of CA-IgM, the cells could bind to the double-coated well more efficiently than to that coated with CA-IgM alone.

Discussion

In the previous studies (Ohori et al., 1986; Fukamizu et al., 1986), we described a new adhesive protein from human plasma. It seemed to have a complicated structure containing subunits that are identical with or related to the heavy chains of IgM, IgG, and IgA. We named this protein cell-binding immunoglobulin-like protein. However, when this protein was further purified, it was found to be a kind of immunoglobulin M, and we now propose the name cell-adhesive IgM for this protein.

Many adhesive proteins contain the RGD sequence as their cell recognition site. The cell-adhesive activity of CA-IgM was inhibited by a RGD-containing peptide, suggesting that this protein also contains the RGD sequence as the cell-binding site, although direct evidence has not yet been obtained. The cell-binding activity of CA-IgM was not inhibited by anti κ or anti λ chain antibody but was inhibited by anti μ and anti Fc antibodies. This suggests that the RGD sequence may be located in the Fc region of the μ chain. This also suggests that the mechanism of the cell binding differs from that of the ordinary antigen–antibody reaction. Ordinary human IgM does not have cell-adhesive activity, and its Fc does not have RGD sequences (Lin & Putnam, 1981). Therefore, CA-IgM seems a special subset of IgM having a unique sequence in the Fc region.

Memberships of the immunoglobulin gene superfamily have been studied actively in recent years (Williams, 1984; Hunkapiller & Hood, 1986). Members of this superfamily have a common structure called the immunoglobulin homology unit, a structure composed of a sequence of about 110 amino acid residues characterized by a centrally placed disulfide bridge that stabilizes a series of antiparallel strands into the socalled antibody fold. They have three kinds of functions: (1) cell recognition such as Thy 1 (Williams & Gagnon, 1982), N-CAM (Cunningham et al., 1987), or MAG (Arquint et al., 1987; Salzer et al., 1987), (2) immune response such as immunoglobulins, and (3) receptors such as poly(Ig) (Mostov et al., 1984). These widely divergent examples indicate evolutional versatility of the immunoglobulin homology for structure and functions. The data described in the present paper suggest that some groups of immunoglobulins have functions for cell recognition and adhesion rather than immunological functions. It should be mentioned that some members of the immunoglobulin gene superfamily, MAG (Salzer et al., 1987) and L1 (Moos et al., 1988), also contain the RGD sequence.

Various extracellular matrix components interact with each other and with other macromolecules. For example, collagens interact with glycosaminoglycans, fibronectin, laminin, and vitronectin (Yamada, 1983). However, only a few biochemical works have been reported on the substances interacting with elastin. One of them is a protein called elastonectin (Hornebeck et al., 1986). The present study demonstrated that CA-IgM binds to elastin and laminin. This suggests that CA-IgM may play a role in many cellular functions as a ligand between cells and elastin and between cells and laminin. Inhibition studies with antibodies indicated that the site for the binding to α-elastin is located in the Fc region. However, the elastin-binding site seems to be different from the cell-binding site, since α-elastin did not inhibit the cell adhesion but rather promoted it. This promotional effect may have physiological importance, although its mechanism is not known. Current experiments are aimed at elucidating the precise structure and binding mechanism of CA-IgM, receptors for CA-IgM on the cell surface, and plasma levels of CA-IgM on different physiological and pathological conditions.

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Independent Analysis of Bait Region Cleavage Dependent and Thiolester Bond Cleavage Dependent Conformational Changes by Cross-Linking of α_2-Macroglobulin with *cis*-Dichlorodiammineplatinum(II) and Dithiobis(succinimidyl propionate)†

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ABSTRACT: Treatment of the human plasma proteinase inhibitor α_2-macroglobulin (α_2M) with proteinase results in conformational changes in the inhibitor and subsequent activation and cleavage of the internal thiolester bonds of α_2M. Previous studies from this laboratory have shown that cross-linking the α_2M subunits with *cis*-dichlorodiammineplatinum(II) (*cis*-DDP) prevents the proteinase-induced conformational changes which lead to the activation and cleavage of the internal thiolester bonds of α_2M. In addition, *cis*-DDP treatment prevents the proteinase- or CH_3NH_2-induced conformational changes in α_2M which lead to a "slow" to "fast" change in nondenaturing polyacrylamide gel electrophoresis. In this paper, we demonstrate that treatment of α_2M with dithiobis(succinimidyl propionate) (DSP) also results in cross-linking of the subunits of α_2M with concomitant loss of proteinase inhibitory activity. Although proteinase is not inhibited by DSP-treated α_2M, bait region specific proteolysis of the α_2M subunits still occurs. Unlike *cis*-DDP-treated α_2M, however, incubation of DSP-treated α_2M with proteinase does not prevent the bait region cleavage dependent conformational changes which lead to activation and cleavage of the internal thiolester bonds in α_2M. On the other hand, cross-linking of α_2M with DSP does prevent the conformational changes which trigger receptor recognition site exposure following cleavage of the α_2M thiolester bonds by CH_3NH_2. These conformational changes, however, occur following incubation of the CH_3NH_2-treated protein with proteinase. These results demonstrate that intersubunit cross-linking by either *cis*-DDP or DSP allows the relative contribution of bait region cleavage dependent and thiolester bond cleavage dependent conformational changes in α_2M to be analyzed separately.

Human α_2-macroglobulin (α_2M)[1] is a proteinase inhibitor present in plasma and other body fluids at concentrations up to 3 μM. α_2M functions as a proteinase inhibitor by a mechanism which has been referred to as "proteinase trapping" (Barrett & Starkey, 1973; Barrett et al., 1979). Since that time, numerous investigators have confirmed this hypothesis and have extended it to explain proteinase inhibition by other, nonhuman α-macroglobulin homologues such as the chicken and duck ovostatins (Nagase & Harris, 1983; Nagase et al., 1983; Feldman & Pizzo, 1984a) and the rat proteins α_2M and α_1M (Gonias et al., 1983). Central to the trap hypothesis is the concept that proteolytic cleavage of α_2M at a "bait region" leads to a series of conformational changes in the inhibitor which sterically inhibit the activity of α_2M-bound proteinase (Harpel, 1973; Barrett & Starkey, 1973; Salvesen & Barrett, 1980). One consequence of this conformational change is that receptor recognition sites become exposed on the inhibitor, which allow the proteinase–inhibitor complex to be rapidly taken up and degraded by a number of cell types (Debanne et al., 1975; Van Leuven et al., 1979; Imber & Pizzo, 1981). The unusual properties of α_2M have been extensively reviewed (Pizzo & Gonias, 1984; Sottrup-Jensen, 1987).

Following proteolytic cleavage of α_2M, a series of conformational changes occur in the inhibitor (Barrett et al., 1979; Gonias et al., 1982; Björk & Fish, 1982) which lead to the

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[1] Abbreviations: α_2M, α_2-macroglobulin; *cis*-DDP, *cis*-dichlorodiammineplatinum(II); DSP, dithiobis(succinimidyl propionate); PAGE, polyacrylamide gel electrophoresis; TNS, 6-(*p*-toluidino)-2-naphthalenesulfonic acid; SDS, sodium dodecyl sulfate; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); α_2M_{DSP}, dithiobis(succinimidyl propionate)-treated α_2M; α_2M_{Pt}, *cis*-dichlorodiammineplatinum(II)-treated α_2M.

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