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A new CD43 monoclonal antibody induces homotypic aggregation of human leucocytes through a CD11a/CD18-dependent and -independent mechanism

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SUMMARY

We describe a monoclonal antibody (mAb), designated 1.C1, that causes rapid and vigorous aggregation among normal leucocytes and among T and myeloid/monocytic cell lines. As shown by competitive binding and sequential immunoprecipitation experiments, the antigen recognized by mAb 1.C1 is a 115,000 MW sialoglycoprotein, that corresponds to the human CD43 antigen, also known as leukosialin or sialophorin. The aggregation process starts within minutes and reaches maximum level 6–18 hr after addition of the antibody. It is dependent on active cell metabolism (inhibited at low temperatures and by a mixture of the metabolic poisons azide and 2-deoxy-D-glucose), a fluid plasma membrane (inhibited by pretreatment of the cells with paraformaldehyde) and an intact cytoskeleton (inhibited by cytochalasin B). Two reference CD43 antibodies (MEM-59 and DF-T1), both binding the same or closely related sialic acid-dependent epitope as mAb 1.C1, are also capable of inducing cell clump formation. CD11a/CD18 mAb block the 1.C1-induced adhesion of resting peripheral blood leucocytes, but not of haematopoietic cell line cells. In addition, mAb 1.C1 induces homotypic aggregation of K-562 cells, which do not express members of the β2 integrin subfamily on their surface. These data suggest that triggering of the CD43 antigen promotes homotypic cell adhesion that is mediated by both CD11a/CD18-dependent and -independent pathways.

INTRODUCTION

CD43 antigen, also known as leukosialin, large sialoglycoprotein, gpL115 or sialophorin, is the major sialoglycoprotein of human leucocytes. It is a highly glycosylated integral membrane protein of apparent molecular weight 110,000–130,000, present on the surface of nearly all human T lymphocytes, monocytes, granulocytes and some B lymphocytes.[1–7] Carbohydrate (one N-linked and 70–90 O-linked carbohydrate chains) contributes by more than 50% to its molecular weight.[1,4] The structure of the O-glycans varies among different cell types[8] and is subject to changes during T-cell activation.[9] Sequence analysis of its cDNA reveals a large intracytoplasmic region of 123 amino acids, a 23-amino acid transmembrane region, and a serine/threonine-rich extracellular domain of 235 residues.[10,11] Homologous molecules have been described in the mouse,[12,13] rat,[14,15] and rabbit.[16]

Monoclonal antibodies (mAb) to the CD43 antigen induce homotypic adhesion of T cells,[17] monocytes[18] and neutrophils.[19] MAb L10 causes monocyte-dependent proliferation of peripheral blood T cells.[20] This activation involves phospholipase C and the release of intracellular Ca^{2+} and proceeds independently of T-cell receptor/CD3-mediated signalling.[21] MAb B1B6 by itself is only weakly mitogenic for T cells, but significantly amplifies T-cell proliferation induced by lectins, phorbol ester, or CD3 mAb, possibly by a mechanism which involves an increased secretion of interleukin-2.[17] Activation of protein kinase C by phorbol 12-myristate 13-acetate (PMA) leads to hyperphosphorylation of the CD43 antigen.[22] Expression of the human CD43 antigen in an HLA-DR-specific murine T-cell hybridoma enhances the antigen-specific response to stimulation by the human lymphoblastoid cell line Daudi.[23] Furthermore, Daudi cells have been shown to bind specifically to purified immobilized CD43 antigen.[23] All these data indicate that leukosialin may be part of a physiological ligand–receptor complex involved in cell activation. This notion is further substantiated by recent evidence indicating that intercellular adhesion molecule-1 (ICAM-1, CD54 antigen) can function as a ligand for the CD43 antigen.[24]

Here we report that a new mAb 1.C1, recognizing a sialic acid-dependent epitope on CD43 antigen, promotes homotypic leucocyte adhesion. The aggregation process not only involves CD11a/CD18 molecules, as was shown previously,[17,18] but also other adhesion structures.

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MATERIALS AND METHODS

Cells and cell lines

Human cell lines (see Table 1) were cultured routinely in RPMI-1640 medium supplemented with 10% heat-inactivated foetal

Table 1. Summary of flow cytometry, Western blotting and aggregation data obtained with mAb 1.C1 on different cell types

Cell line	Type*	Expression† of 1.C1 antigen	M_r‡	Aggregation§ Without mAb	With mAb 1.C1 (5 μg/ml)
Myeloid/monocytic cell lines					
KG-1	AML	3+	ND¶	0	4+
KG-1a	AML	3+	110,000	1+	4+
HL-60	APL	2+	130,000	0	5+
U937	AMoL	3+	130,000	0	5+
THP-1	AMoL	3+	ND	1+	3+
Erythroleukaemic cell lines					
K-562	CML	2+	115,000	2+	4+
HEL	CML	2+	125,000	2+	4+
T-cell lines					
CCRF-CEM	ALL	3+	ND	0	4+
Jurkat	ALL	2+	120,000	1+	2+
MOLT-4	ALL	2+	135,000	2+	4+
HuT 78	CL	2+	125,000	4+	4+
B-cell lines					
Daudi	BL	0	ND	3+	3+
Raji	BL	1+	115,000	3+	3+
CCRF-SB	ALL	2+	115,000	5+	5+
Normal peripheral blood					
Lymphocytes		3+	ND	0	3+
Neutrophils		2+	ND	0	3+
Monocytes		2+	ND	ND	ND
Erythrocytes		0	ND	ND	ND
Platelets		±	ND	ND	ND

* Type of cell lines were: AML, acute myelogenous leukaemia; APL, acute promyelocytic leukaemia; AMoL, acute monocytic leukaemia; CML, chronic myelogenous leukaemia; ALL, acute lymphoblastic leukaemia; CL, cutaneous T-cell lymphoma; BL, Burkitt lymphoma.

† Presence or absence of 1.C1 antigen was determined by indirect immunofluorescence analysed on the FACScan. Flow cytometry score is based on: mean fluorescence intensity obtained with mAb 1.C1 divided by mean fluorescence intensity obtained with irrelevant mAb 1.24. When this figure is below 1·5 (score 0), between 1·5 and 2 (score ±), between 2 and 10 (score 1+), between 10 and 50 (score 2+), more than 50 (score 3+).

‡ As determined by Western blotting of cell extracts run on 7·5% SDS–PAGE under non-reducing conditions.

§ Aggregation score, as described in Materials and Methods, read after 18 hr.

¶ ND, not done.

calf serum (FCS), 2 mM L-glutamine, 100 IU/ml penicillin, and 100 μg/ml streptomycin (all reagents from Gibco, Ghent, Belgium). Cells were pelleted and washed three times in Ca^{2+}/Mg^{2+}-free Dulbecco's phosphate-buffered saline (D'PBS) before use in radiolabelling and homotypic aggregation experiments. Peripheral blood mononuclear cells (PBMC) were isolated from anti-coagulated venous blood of healthy volunteers by density gradient centrifugation over Lymphoprep (Nycomed, Oslo, Norway). Neutrophils were collected from the pellet after 3% dextran sedimentation and hypotonic lysis of contaminating red blood cells. Erythrocytes were prepared by removing the buffy coat after centrifugation of whole blood.

mAb

The 1.C1 hybridoma is a product of the fusion of SP2/0-Ag14 mouse myeloma cells with splenocytes from a BALB/c mouse immunized by intraperitoneal injection of human KG-1a cells. Immunization, fusion, selection and cloning protocols were essentially as described prevously.[25] Hybridoma supernatants were screened for reactivity with KG-1a cells by indirect immunofluorescence. MAb 1.C1 is of the IgG1κ subclass as determined with a mAb isotyping kit, making use of strips coated with rat anti-mouse isotype-specific antibodies (Innogenetics, Antwerp, Belgium). After purification from ascitic fluid by protein G-Sepharose column chromatography (Pharmacia, Uppsala, Sweden), mAb 1.C1 was biotinylated using D-biotinyl-ε-aminocaproic acid-*N*-hydroxysuccinimide ester (Boehringer, Mannheim, Germany) according to the instructions of the manufacturer. 2.E11 (IgG1κ), recognizing a monomorphic determinant on human major histocompatibility complex class I heavy chain(s), is a mAb isolated from the same immunization and screening that produced mAb 1.C1 (W. De Smet, H. Walter and L. Van Hove, unpublished observation). MAb 1.24 (IgG1) is directed against a rabbit leucocyte surface

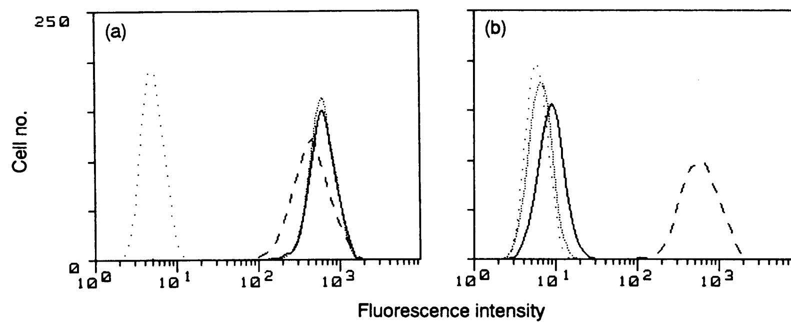

Figure 1. FACS histogram of mAb 1.C1 (——), MEM-59 (········), 2.E11 (– – –) and 1.24 (· · · ·) binding on KG-1a cells before (a) or after (b) treatment with neuraminidase. The epitope recognized by mAb 1.C1, MEM-59 (and DF-T1, results not shown) is destroyed by neuraminidase, while the 2.E11 epitope is not.

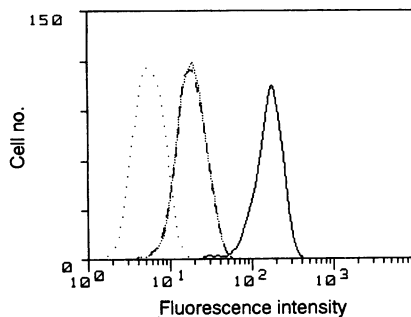

Figure 2. Binding of biotinylated mAb 1.C1 to KG-1a cells in the presence of the following inhibitor mAb: 1.C1 (– – –), DF-T1 (········) or 2.E11 (——). Binding curves with or without control inhibitor mAb are identical and represented by the same profile (——). Background curve (streptavidin–FITC alone without biotinylated mAb in the first step) is also shown (· · · ·). Binding of biotinylated mAb 1.C1 is inhibited by itself, by DF-T1 (and MEM-59, results not shown), but is not influenced by 2.E11.

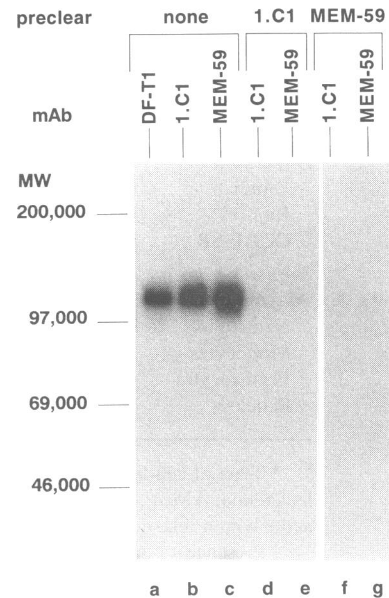

Figure 3. Immunoprecipitation of CD43 antigen by mAb 1.C1. Radiolabelled material from KG-1a extracts either was not (lanes a–c) or was precleared with mAb 1.C1 (lanes d, e) or MEM-59 (lanes f, g), respectively, before precipitation with either mAb DF-T1 (lane a), 1.C1 (lanes b, d and f) or MEM-59 (lanes c, e and g), and analysis on a 7·5% non-reducing SDS–PAGE. Positions of MW marker proteins are indicated on the left.

antigen and does not cross-react with human cells.[25] MAb MEM-59 (IgG1κ) (obtained from Dr V. Hořejši, Institute of Molecular Genetics, Prague, Czechoslovakia)[26] and DF-T1 (obtained from Dr D. Flavell, Southampton General Hospital, Southampton, U.K.)[27] are both reference CD43 antibodies.[7] Antibodies against the α-(CLB-LFA-1/2, CD11a, IgG1)[28] and β-chain (CLB-LFA-1/1, CD18, IgG1)[29] of lymphocyte function-associated antigen-1 (LFA-1) were purchased from Janssen Biochimica (Beerse, Belgium). Anti-ICAM-1 (84H10, CD54, IgG1)[30] mAb was obtained from Immunotech (Marseilles, France).

Flow cytometry

Aliquots of 5×10^5 cells (in 100 μl D'PBS/2% FCS/0·02% NaN_3) were incubated for 30 min at 4° with a saturating amount of mAb, washed once, incubated for another 30 min with fluorescein isothiocyanate (FITC)-labelled affinity-purified goat $F(ab')_2$ anti-mouse Ig (Tago, Burlingame, CA) and washed again. The stained cells (except erythrocytes which were analysed immediately) were then fixed with 0·5% paraformaldehyde in D'PBS and analysed on a FACScan (Becton-Dickinson, Erembodegem, Belgium). Where applicable, different cell populations (e.g. lymphocytes, monocytes, neutrophils) were identified based on two-dimensional light scatter characteristics. Heat-inactivated normal rabbit serum was added in both the first and second incubation step to avoid aspecific Fcγ receptor staining. For competition binding, KG-1a cells were incubated with unlabelled mAb (30 min, 4°), washed and incubated with an appropriate dilution of biotinylated 1.C1 (30 min, 4°). After washing, streptavidin–FITC (2 μg/test) (Boehringer) was added for another 30 min at 4°. After washing and fixation, cells were subjected to flow cytometric analysis.

Radiolabelling of cell surface proteins, immunoprecipitation, gel electrophoresis and autoradiography

Cells were labelled at the surface by lactoperoxidase-catalysed iodination, as described previously.[31] Radioiodinated cells were lysed with Nonidet P-40 (NP-40) lysis buffer (50 mM Tris–HCl pH 8·0, containing 150 mM NaCl, 0·5% NP-40, 100 mM iodoacetamide, 3 mM EDTA, 1 μM pepstatin A, 0·03 TIU/ml aprotinin, 2 mM PMSF and 0·1% NaN_3). After 15 min at 4°, the

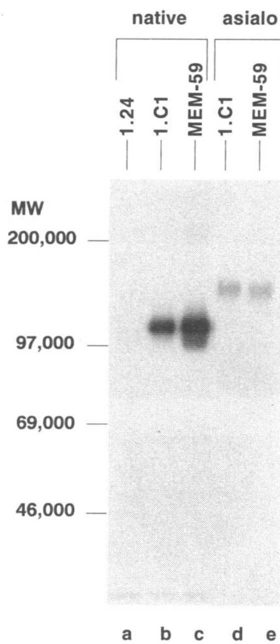

Figure 4. Apparent molecular weight of 1.C1 antigen changes drastically after neuraminidase treatment. Radiolabelled material from KG-1a extracts was precipitated with mAb 1.24 (lane a), 1.C1 (lanes b and d) or MEM-59 (lanes c and e). The precipitates were analysed with (lanes d and e) or without (lanes a–c) neuraminidase treatment by SDS–PAGE (7·5% acrylamide, reducing conditions).

extract was centrifuged at 30,000 *g* for 1 hr. The resulting supernatant was preadsorbed with protein A–Sepharose (Pharmacia) for 2 hr at 4°. Fifty microlitres ($2–3 \times 10^6$ cell equivalents) of lysate were mixed with 10–50 μl of appropriately diluted mAb and incubated overnight at 4°. The antigen–antibody complexes formed were precipitated with 150 μl of 10% protein A–Sepharose (3 hr, 4°), washed three times in lysis buffer containing 0·5% deoxycholate and 0·1% bovine serum albumin (BSA), disrupted in sample buffer (4 min, 95°) and analysed by one-dimensional SDS–PAGE according to Laemmli.[32] After Coomassie staining and drying, the gels were subjected to autoradiography at −80° with X-ray film and intensifying screens.

Neuraminidase treatment of cells and immunoprecipitates
Neuraminidase (from *Arthrobacter ureafaciens*) (Boehringer) treatment of KG-1a cells (1×10^7 cells/ml) was performed in RPMI-1640 medium with an enzyme concentration of 0·1 U/ml for 45 min at 37°. Immunoprecipitates were treated with 0·1 U/ml for 120 min at 37° in a buffer containing 0·2% SDS, 1% NP-40, 5 mM calcium carbonate and 20 mM sodium cacodylate, pH 6·5.

Aggregation assay
Cells ($1–2 \times 10^5$ cells in 200 μl RPMI-1640 medium containing 10% FCS) seeded in 96-well flat-bottomed microtitre plates (Costar, Cambridge, MA) were incubated for various time periods at 37° in a 5% CO_2 incubator (or at 4° or 20°) in the presence of mAb 1.C1 or PMA. Cells were preincubated with various inhibitors of cell metabolism (or blocking mAb CLB-LFA-1/1, CLB-LFA-1/2 and 84H10) for 30 min at 37° before adding mAb 1.C1 or PMA. Homotypic aggregation was measured in a semi-quantitative manner by minor modification of the method described by Rothlein and Springer.[33] Scores range from 0 to 5+, where 0 indicates that essentially no cells are aggregated in clusters; 1+ = <10% of the cells found in clusters; 2+ = 10–50% of the cells aggregated in small clumps; 3+ = 50–80% of the cells aggregated in medium-sized clumps; 4+ = >80% of the cells found in large aggregates; 5+ = nearly 100% of the cells found in very large compact aggregates. Photographs were taken at ×125 on an Olympus inverted microscope.

RESULTS

mAb 1.C1 belongs to cluster CD43

A number of B, T, myeloid and erythroid cell lines and normal blood cells were tested by flow cytometry for their reactivity with mAb 1.C1 (Table 1). The antigen, defined by mAb 1.C1, obviously is a non-lineage antigen present in moderate to large amounts on nearly all cell types tested. In contrast, it is absent from erythrocytes and expressed at very low level on platelets. As has been reported for CD43 mAb,[7] mAb 1.C1 reacts poorly or does not react at all with Burkitt lines such as Daudi and Raji. MAb 1.C1, as the two other reference CD43 mAb MEM-59 and DF-T1, is directed against a neuraminidase-sensitive epitope (Fig. 1). Preincubation of KG-1a cell suspensions with MEM-59 or DF-T1 blocks the binding of biotinylated 1.C1 (Fig. 2).

Immunoprecipitation and SDS–PAGE analysis of the ^{125}I-labelled 1.C1 antigen on KG-1a cells results in a band of apparent molecular weight 110,000 when 7·5% gels were run both under reducing or non-reducing conditions (Fig. 3). Further studies reveal that the apparent molecular weight of 1.C1 antigen: (1) is dependent on the percentage of acrylamide used in the gel (results not shown); (2) is dramatically altered after neuraminidase treatment of the precipitate (Fig. 4); and (3) varies according to the cell line tested (Table 1). Similar observations have previously been reported for leukosialin[1] and CD43 antigen.[26,27] Paired and crossed immunoprecipitation experiments were then performed to determine whether mAb 1.C1, MEM-59 and DF-T1 recognize the same cell surface glycoprotein. In ^{125}I-labelled KG-1a extracts, all these antibodies precipitate a band of identical apparent molecular weight (Fig. 3). In addition, preclearing with mAb 1.C1 is shown to remove almost all MEM-59 reactivity, and vice versa (Fig. 3).

mAb 1.C1 induces homotypic cell aggregation

A prominent feature of mAb 1.C1 is its ability to induce rapid and vigorous homotypic cell aggregation in most of the cell lines studied (Table 1, Fig. 5). Notable exceptions are the B-cell lines Daudi, Raji and CCRF-SB, which are not influenced by the presence of the antibody. However, in our hands, these cell lines grow spontaneously in large aggregates, so that the effect of mAb 1.C1 is difficult to evaluate. Both mAb DF-T1 and MEM-59, directed against the same or spatially proximal CD43 epitope as 1.C1 (see above), similarly stimulate homotypic cell interactions (results not shown). Time-kinetics studies show that mAb 1.C1-induced aggregation occurs quite rapidly, with small aggregates of cells visible within 15 min of culture, and much larger aggregates evident in 1–2 hr. The kinetics of aggregation is faster than with PMA, reaching plateau levels

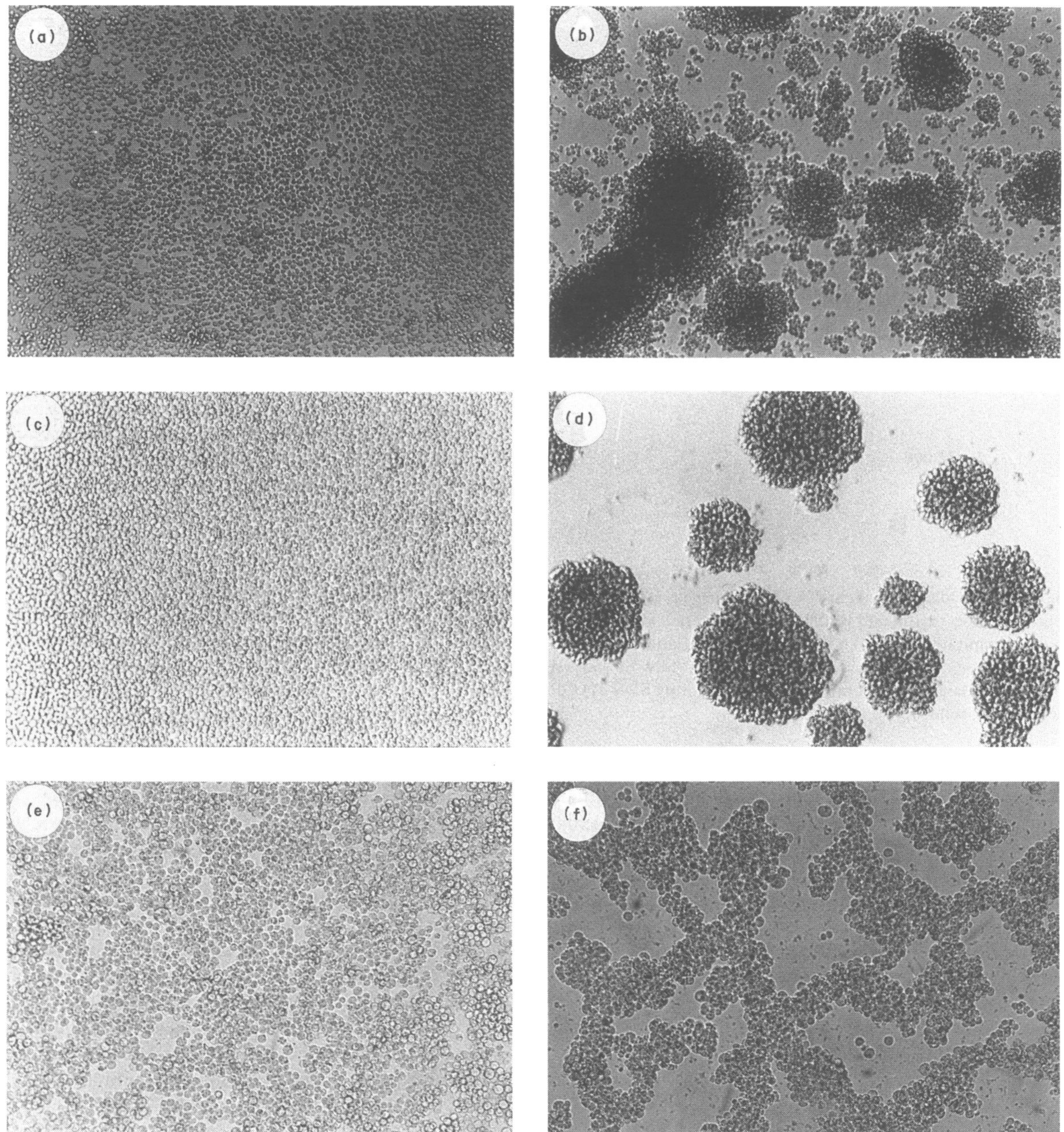

Figure 5. Spontaneous and 1.C1-induced aggregation of human cell lines. KG-1a (a, b), U937 (c, d) and K-562 (e, f) cells were incubated for 20 hr at 37° in the presence of 5 μg/ml mAb 2.E11 (anti-HLA class I) (or exposed to medium alone) (a, c, e) or 5 μg/ml mAb 1.C1 (b, d, f). Original magnification ×125.

after 10 hr of incubation, the time at which the PMA-induced aggregation is about to start (results not shown).

Specificity of mAb 1.C1-induced aggregation

The specificity of the aggregation induced by 1.C1 is illustrated by the fact that mAb directed against several other major cell surface molecules known to be present on human cell lines (including HLA class I, CD44, CD45, CD45RA and CD71 antigens) have no effect (Fig. 5 and results not shown). All these mAb are of the same isotype as 1.C1, and therefore control for possible involvement of Fcγ receptor in the phenomenon. In addition, aggregation responses were seen with cell lines (e.g. CCRF-CEM, MOLT-4) lacking Fcγ receptor. Moreover, the addition of 100-fold higher amounts of heat-aggregated normal rabbit Ig fails to inhibit 1.C1-induced clumping. Pretreatment of the cells with paraformaldehyde, although not destroying the 1.C1 epitope, prevents mAb 1.C1-mediated adhesion.

mAb 1.C1-induced homotypic cell aggregation is energy dependent and requires an intact cytoskeleton

Phorbol ester-induced homotypic aggregation is LFA-1-mediated and dependent on divalent cations and an intact cytoskeleton.[33] A number of experiments were performed to investigate whether the homotypic aggregation induced by mAb

Table 2. Summary of physiological requirements of mAb 1.C1 and PMA-induced homotypic aggregation

	Aggregation			
	U937		CCRF-CEM	
Treatment*	+1.C1	+PMA	+1.C1	+PMA
37°	4+	3+	3+	2+
20°	3+	1+	3+	0
4°	0	0	0	0
4°→37°	4+	3+	3+	2+
50 mM 2-deoxy-D-glucose+0·1% NaN_3	0/1+	0	0/1+	0
20 μM cytochalasin B	0/1+	0	0/1+	0
1–10 mM EDTA	4+	0/1+	3+/4+	0
1–10 mM EGTA	4+	3+	3+/4+	1+/2+
0·5% paraformaldehyde†	0	ND‡	0	ND

* Cells were washed three times in Ca^{2+}/Mg^{2+}-free D'PBS. They were then incubated (in RPMI with 10% FCS) with various inhibitors of cell metabolism for 30 min at 37° before adding mAb 1.C1 (1 μg/ml) or PMA (20 ng/ml). Aggregation was scored after 5 hr (in the case of CCRF-CEM) or 18 hr (U937).

† Cells were pretreated with 0·5% paraformaldehyde (60 min, 4°) and washed several times before addition of 1.C1.

‡ ND, not done.

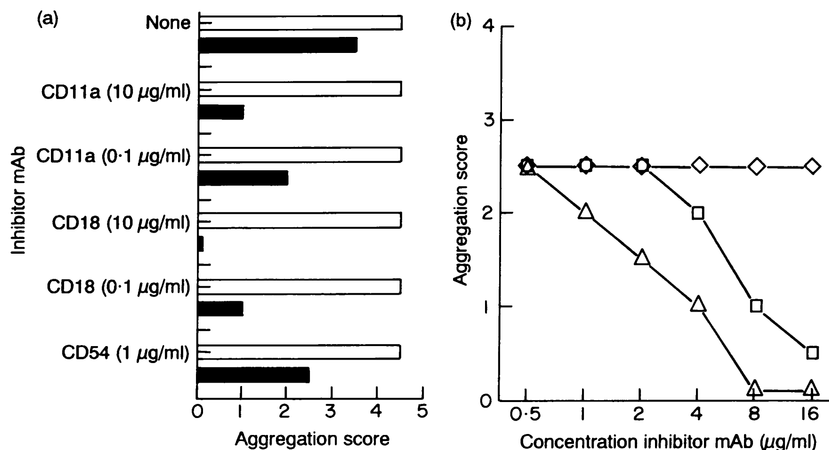

Figure 6. (a) U937 cells were cultured in normal medium or with the indicated blocking mAb for 30 min at 37° before the addition of optimal concentrations of either mAb 1.C1 (2 μg/ml) (□) or PMA (20 ng/ml) (■). Aggregation is scored after 20 hr of additional incubation. CD18 mAb CLB-LFA-1/1 and to a lesser extent CD11a mAb CLB-LFA-1/2 are able to block PMA- but not 1.C1-induced aggregation. (b) KG-1a cells were incubated with various concentrations of blocking mAb 2.E11 (◇), CLB-LFA-1/2 (□) or CLB-LFA-1/1 (△) for 30 min at 37° before addition of 100 ng/ml mAb 1.C1. Aggregation is scored after 20 hr of additional incubation. CD11a/CD18 mAb show a dose-dependent inhibition of KG-1a cell aggregation induced by suboptimal concentrations of 1.C1.

1.C1 has simlar requirements (Table 2). Low temperature completely inhibits both types of aggregation, but returning the cells to 37° allows aggregation to proceed at a normal rate. While blocking PMA-induced adhesion, room temperature has no or weak inhibitory effects on 1.C1-induced aggregation (depending on the cell type tested). Treatment of the cells with cytochalasin B, which interferes with the formation of microfilaments, also blocks aggregation. Sodium azide and 2-deoxy-D-glucose have weak to moderate effects when tested alone (depending on the cell type tested) (results not shown), but a mixture of these metabolic poisons always completely inhibits aggregation. Surprisingly, the presence of Mg^{2+} and/or Ca^{2+} cations is not necessary for 1.C1-triggered homotypic adhesion. The addition of EDTA does not prevent 1.C1-induced aggregation but, as expected, abrogates PMA-induced aggregation. Studies determining cation dependency were also carried out in

Hanks' balanced salt solution (HBSS) or HBSS deficient in Ca^{2+}/Mg^{2+} (and supplemented with 2% BSA and 2 mg/ml D-glucose) with similar results.

CD11a/CD18-dependent and -independent mechanisms of 1.C1-induced homotypic adhesion

Antibodies CLB-LFA-1/2 and CLB-LFA-1/1, directed respectively against the α- and β-chain of the LFA-1 complex, block cell aggregation of resting PBMC (results not shown). Homotypic aggregation of haematopoietic cell lines in response to suboptimal concentrations of mAb 1.C1 (≤100 ng/ml) is likewise eliminated by both antibodies (Fig. 6b). In contrast, adhesion induced by optimal concentrations of mAb 1.C1 (≥1 μg/ml) on cell line cells (example given for U937 cells in Fig. 6a) is not affected by amounts of CD11a/CD18 mAb sufficient to inhibit completely PMA-induced homotypic aggregation of the same cells (Fig. 6a). These findings suggest that other adhesion receptors, in addition to LFA-1, may participate in 1.C1-induced homotypic aggregation. This notion is further substantiated by the fact that mAb 1.C1 also induces clumping of K-562 cells (Table 1, Fig. 5), which do not express members of the β2 integrin subfamily on their surface (ref. 34 and results not shown). As is the case for CCRF-CEM and U937 cells (Table 2), the adhesion of K-562 cells is resistant to treatment with EDTA, again indicating that it does not involve any of the known leucocyte integrins.[35]

DISCUSSION

The function of the CD43 molecule (leukosialin) has not, thus far, been elucidated. Pro-adhesive[24] as well as anti-adhesive[36] features have been attributed to it. In this study, we have used a new CD43 mAb, designated 1.C1, to explore the role of leukosialin in cell adhesion.

The following flow cytometry and immunochemical data indicate that mAb 1.C1 belongs to cluster CD43 and recognizes the same (or spatially related) neuraminidase-sensitive epitope as reference CD43 mAb DF-T1 and MEM-59: (1) mAb 1.C1 and MEM-59 almost completely clear each others antigen in crossed immunoprecipitations; (2) the binding of biotinylated 1.C1 is blocked by mAb MEM-59 and DF-T1; and (3) the binding of all three mAb is abrogated by treatment of the cells with neuraminidase. This latter observation does not necessarily imply that the mAb are directed against carbohydrate determinants. In the case of rat leukosialin, the conformation or accessibility of linear protein epitopes has been shown to be modified by glycosylation.[15] MAb, whose epitopes were affected by neuraminidase treatment of rat leukosialin, clearly reacted with the extracellular domain of the antigen expressed in an unglycosylated form in *Escherichia coli*.[15]

MAb 1.C1 causes rapid and vigorous aggregation among normal leucocytes and among myeloid/monocytic and T-lymphoid leukaemic cell lines. CD11a/CD18 mAb block 1.C1-induced aggregation of resting PBMC. This is in keeping with earlier results indicating that CD43-induced homotypic adhesion of peripheral blood T lymphocytes,[17] monocytes[18] and neutrophils[19] is mediated through LFA-1 molecules. However, in contrast with normal resting PBMC, adhesion induced by mAb 1.C1 among cell line cells (KG-1a, U937, CCRF-CEM) is not affected by CD11a/CD18 mAb. This suggests that CD43-induced homotypic adhesion of cell line cells, which probably are already in a state of activation may be LFA-1 independent. Similar observations have been described for the B- cell-specific CD19 marker. LFA-1-dependent mechanisms of adhesion operate in normal tonsillar B cells stimulated with CD19 mAb, while LFA-1-independent mechanisms predominate in B cells activated with a mixture of PMA and CD19 mAb or in B-cell lines.[37] Alternatively, as has been shown for anti-HLA-D mAb or CD4/Ig heavy chain fusion proteins, the LFA-1-mediated adhesion pathway may be activated during the initial stages of cell interaction but may subsequently be taken over by additional LFA-1-independent pathways.[38] Such a mechanism has been described for neutrophil aggregation induced by CD43 mAb BS-1.[19] In agreement with this hypothesis, we have seen that CD11a/CD18 mAb inhibit homotypic aggregation of KG-1a cells when it is induced by low concentrations (≤100 ng/ml) of mAb 1.C1 (Fig. 6b). However, this inhibition is not always observed and seems to depend on the cell cycle stage and on the number of cells used in the aggregation assay (inhibition is more prominent when the cells are used early after serial passage) (results not shown). The existence of the LFA-1-independent pathway is further corroborated by the fact that 1.C1-induced aggregation is also observed (Fig. 5, Table 1) with K-562 cells (which lack surface CD11a/CD18 molecules) (ref. 34 and results not shown).

There are two possible explanations for the adhesive effect induced by CD43 mAb. Firstly, binding of CD43 mAb (thought to substitute for one of the ligands for the CD43 antigen) triggers a signal that activates other (CD11a/CD18-dependent and -independent) adhesion pathways. Secondly, binding of CD43 mAb induces a conformational change in leukosialin that allows it to participate in self–self adhesion or to react with an (unidentified) counter-receptor on adjacent cells or in the extracellular matrix. It has recently been shown that ICAM-1 can function as a counter-receptor for CD43 antigen.[24] Thus, the 1.C1-mediated aggregation observed in this study could be due to the interaction of CD43 and CD54 antigens on opposite cells. Our preliminary data indicate the existence of alternative cellular ligands for leukosialin, because: (1) aggregation can be induced in cell lines which express little or no ICAM-1[39] such as THP-1 and HEL (Table 1); (2) addition of CD54 mAb 84H10 has no inhibitory effect on 1.C-induced aggregation of U937 cells (Fig. 6a). However, mAb 84H10 had only weak blocking capacity when tested in parallel on PMA-induced aggregation. Therefore, other mAb (directed preferentially against different epitopes of the CD54 molecule) or synthetic peptide analogues should be tested before a firm conclusion can be reached.

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Note added in proof

Cyster and Williams recently proposed another mechanism to explain the phenomenon of CD43-induced homotypic aggregation.* According to their hypothesis, the clustering process is initiated by the CD43 antibody (providing a cross-linking effect between cells that overcomes repulsion) and subsequently completed by other adhesion molecules. Our results cannot exclude this elegant possibility as we have not tested Fab fragments of mAb 1.C1.

* Cyster J.G. & Williams A.F. (1992) The importance of cross-linking in the homotypic aggregation of lymphocytes induced by anti-leukosialin (CD43) antibodies. *Eur. J. Immunol.* **22**, 2565.