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Antigenic relationship between the alpha-chain of C3, a leucocyte-surface antigen involved in the activation of phagocytic cells, and a 50,000 MW B-cell antigen

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Summary. A monoclonal antibody, M522, reacting with human monocytes, neutrophils and a proportion of non-adherent PBL in a pattern similar to OKM-1 and anti-Mo-1, and pecipitating a dimer of MW 165,000 and 100,000 from neutrophils, was shown to react with C3 at an epitope localized on the alpha-chain of C3.

$F(ab)_2$- fragments of M522 stimulate the respiratory burst of neutrophils and monocytes. M522 differs from two monoclonal antibodies against the Mo-1 molecule with respect to the capacity to inhibit the binding of sheep erythrocytes coated with different C3-fragments to C-receptor carrying cells. It inhibited the binding of H-coated particles to B-lymphoid cells and precipitated a 50,000 MW molecule from RAJI cells and tonsil lymphocytes.

The results obtained suggest an antigenic relationship between the alpha-chain of C3, the heavy chain of a membrane molecule involved in neutrophil/monocyte activation, and a B-cell molecule of MW 50,000.

Abbreviations: B, factor B of the alternative pathway of complement; BSA, bovine serum albumin; C1, C2, C3, C4, C5, C6, C7, C8 and C9, First to ninth component of complement; C3b, C3bi, C3c and C3d, b, bi, c, d fragment of C3; EAC3b, EAC3bi, EAC3d: sheep red blood cells coated with amboceptor, C1, C4, C2 C3b or C3bi andC3d, respectively; EAC3b-H, EAC3b carrying H; E-H, sheep red blood cells carrying H attached by $CrCl_3$ method; E-M522, sheep red blood cells carrying M522 attached by $CrCl_3$ method; H, formerly β1H, C3b-inactivator-cofactor; I, C3b-inactivator.

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INTRODUCTION

In recent years, several of the membrane molecules involved in the activation of human neutrophils and monocytes have been identified. One of these structures, the molecule recognized by monoclonal antibodies Mac-1, OKM-1, anti-Mo-1 has been shown to contain two polypeptide chains of MWs 165,000 and 95,000, and to share this 95,000 chain with two other leucocyte-membrane molecules LFA-1 and gp 150, 95 (Sanchez-Madrid *et al.*, 1983; Todd & Schlossmann, 1983). This molecule, which in immunofluorescence can be detected on human neutrophils, monocytes/macrophages and natural killer cells (Springer *et al.*, 1979; Ho & Springer, 1982), seems to serve as receptor for iC3b as shown by both inhibition studies with monoclonal antibodies (Beller, Springer & Schreiber, 1982; Arnaout *et al.*, 1983) and by studies demonstrating the binding of the Mo-1 molecule to iC3b (Wright *et al.*, 1983). Its importance in the phagocytic function of neutrophils and monocytes is stressed by the observation that patients deficient in this molecule are prone to frequent infections and that their granulocytes show a wide range of defects observable *in vitro*, such as decreased ingestion of iC3b-coated particles and of some IgG-coated particles (Arnaout *et al.*, 1982; Dana *et al.*, 1983, 1984).

The presence on human leucocytes of some of the complement components found in serum has been reported by several groups. Molecules antigenically related to C1q, C3, C4, C5, C6, C7, C8, C9, B, P and factor H have been found on human B lymphocytes (Osther & Dybkjaer, 1974; Burns & Cawley, 1979; Curd *et al.*, 1978; Wilson *et al.*, 1982). In the case of factors B and H, the molecular weight of the membrane molecule from B cells corresponds to the molecular weight of the serum molecule (Woo & Lachmann, 1981; Schulz *et al.*, 1984). Evidence for the existence of C3 on monocytes (Burns & Cawley, 1979) and of the C5–9 neoantigen on T lymphocytes and monocytes has also been reported (Sundsmo, Kolb & Müller-Eberhardt, 1978). Whether their presence on cell membranes serves any function is not quite clear, although we and others have recently been able to provide evidence that membrane-associated factor H and factor I participate in the binding of C3-coated particles to B lymphocytes (Scheiner *et al.*, 1983; Schulz *et al.*, 1984; Lambris, Dobson & Ross, 1980). An earlier finding involved membrane-associated C4 in the human mixed-lymphocyte reaction (Ferrone, Pellegrino & Cooper, 1976; Burger & Shevach, 1979). Membrane-associated C5 has been implicated in the stimulation of monocytes and lymphocytes (reviewed in Sundsmo, 1982).

A few years ago, Lohmeyer *et al.* (1981) reported a monoclonal antibody, M522, raised against a chronic lymphocytic T-cell leukaemia which was found to react in immunofluorescence with monocytes, neutrophils and 9–17% of non-adherent peripheral blood leucocytes comprising the subset of cells with NK-activity. Its cellular distribution thus corresponds to that of monoclonal antibodies directed against the Mo-1 antigen.

In this study, we found that M522 reacts with the alpha-chain of C3, and that its $F(ab)_2$ fragments stimulate the respiratory burst of human monocytes and neutrophils. It precipitates a dimer of MW 165,000 and 100,000 from monocytes and neutrophils reacting with an epitope on the 165,000 MW chain. These findings could therefore suggest an antigenic relationship between the alpha-chain of C3 and the alpha-chain of a member of the family of leucocyte antigens previously described, possibly the Mo-1 antigen. In addition, M522 differed from two monoclonal antibodies to Mo-1 by only inhibiting the binding of EAC3b-H to RAJI cells and tonsil interphase cells, and it precipitates a 50,000 MW molecule from these two cell types. Results of experiments investigating the relationship of this molecule to the receptor for H are presented.

MATERIAL AND METHODS

Buffers

The following buffers were used: phosphate-buffered saline (PBS) (140 mM NaCl, 10 mM sodium phosphate, pH 7·2); veronal-buffered saline containing EDTA (VBS-EDTA) (140 mM NaCl, 5 mM Na-5,5-diethylbarbiturate, 20 mM EDTA, 0·2% gelatin, pH 7·2); substrate buffer (10 mM potassium phosphate buffer, pH 6·0); PBS containing 0·05% Tween 20 (SERVA, Heidelberg, W. Germany) (PBS-Tween), pH 7·4; PBS containing 0·2 g $CaCl_2$, 0·2 g KCl, 0·04 g $MgCl_2$ and 1 g glucose in 1 litre PBS.

Cells

The cell line RAJI was cultured in RPMI 1640 (Flow, Bonn, W. Germany) supplemented with glutamine (2 MM), 10% FCS, penicillin (100 U/ml), streptomycine (100 μg/ml) and fungizone (2·5 μg/ml). Tonsil interphase cells were prepared as described previously (Schulz *et al.*, 1984). Human neutrophils and monocytes were prepared from 40 ml of freshly drawn heparinized blood. After density-gradient centrifugation on Ficoll-Hypaque, the interphase cells were used as a source of monocytes and the pellet (red cells and PMN) was resuspended in PBS-glucose containing 2% gelatine (Sigma, Munich, W. Germany) and the red cells allowed to sediment for 45 min at 37°. The supernatant containing the neutrophils was removed centrifuged at 400 ***g*** for 15 min, and the remaining red cells lysed in 0·84% NH_4Cl for 10 min at 37°. The neutrophils were then washed twice in PBS-glucose and resuspended in either PBS-glucose or VBS-EDTA at 2×10^6/ml. Sheep erythrocytes were coated with antibodies C1, C4, oxidized C2 andC3b (EAC1423b) and the preparation of EAC3b-H, as well as the conversion to EAC1423bi and EAC1423d, was performed as described by Schmitt, Mussel & Dierich (1981). Sheep red blood cells were coated with purified M522 or H according to Ling, Bishop & Jefferis (1977), using 100 μl M522 or H (1 mg/ml) in 0·9% NaCl and 100 μl 0·025% $CrCl_3$ per 100 μl packed sheep red blood cells.

Antibodies

The monoclonal antibody M522 was described previously (Lohmeyer *et al.*, 1981). In all experiments,

antibody purified from ascites by chromatography on S200 was used. $F(ab)_2$ fragments were prepared by digestion with pepsin. The antibody to Mo-1, VIM-12 was a kind gift from Dr W. Knapp, (Vienna), and OKM-1 was purchased from Ortho Pharmaceuticals, Raritan, NJ. Antibody 111 to C3c (Burger *et al.*, 1982) was a gift from Dr Burger (Heidelberg, W. Germany).

Purification of C components and C3 fragments

C3 was purifed as described by Hammer *et al.* (1981). Any residual H, C5 and IgG was removed by affinity chromatography using sepharose 4B-coupled anti-H, anti-C5, anti-IgG. Purification by preparative SDS-PAGE of C3c and C3d obtained after cleavage with elastase, of alpha- and β-chain obtained after reduction with 2% mercaptoethanol, and of the 40,000 MW C-terminal and 28,000 MW N-terminal elastase-derived fragments of the alpha-chain of C3b has been described elsewhere (Lambris *et al.*, 1984).

C1 was purified from guinea-pig serum according to Colten, Borsos & Rapp (1969) by zonal ultracentrifugation. The protocol of Sim & DiScipio (1982) with some modifications (Alsenz *et al.*, 1984) was used to purify factor H. Purification of P, B was also performed as described. Functionally pure C2, C4, C5, C6, C7, C8 and C9 were purchased from Cordis (Miami, FL). Tritiated H was a gift from Dr J. Lambris (La Jolla, CA) and had a specific activity of 5×10^4 c.p.m./μg.

ELISA

Different complement components and C3 fragments were attached to ELISA plates (Nunc, Copenhagen, Denmark) in the concentrations indicated in 0·1 M $NaHCO_3$, pH 9·6, (50 μl/well) for 2 hr. After saturation with PBS containing 1% BSA, 20 μl M522 (20 μg/ml), VIM12 (20 μg/ml) or OKM-1 (1:100 dilution) were added and the plate incubated at room temperature for 30 min. Detection of bound monoclonal antibody was then performed as described (Schulz *et al.*, 1984).

Rosette inhibition

The rosette inhibition assay has been described elsewhere (Schreiner *et al.*, 1983).

Chemiluminescence

One hundred μl of neutrophils in PBS-glucose (2×10^6/ml) or peripheral blood interphase cells (as a source of monocytes) in RPMI (2×10^6/ml), kept on ice, were mixed with 100 μl Luminol (Serva, Heidelberg, FRG) in PBS or RPMI (0·1 mg/ml). Whereas neutrophils were preincubated at 37° for 15 min, monocytes were used immediately. The reaction was started by adding 100 μl of M522-F $(ab)_2$, 669G2, a monoclonal antibody reacting with neutrophils, kindly donated by Dr Engelberger (Mainz, W. Germany), 40% NH_4SO_4-precipitate of ascites from a non-producer cell line or factor H (all at 50 μg/ml) and the resulting chemoluminescence was measured for 20 seconds every 3 min (monocytes) or 4 min (neutrophils) in a Bioluminat (Berthold, W. Germany).

Radioactive labelling of cells

Labelling by ^{125}I was performed as follows: 10^8 cells in 1 ml PBS were mixed with 100 μl lactoperoxydase (Sigma) (2 mg/ml in PBS), 25 μl 0·003% H_2O_2 and 1 mCi ^{125}I (Amersham, Bucks) and incubated at room temperature for 20 min. Addition of H_2O_2 was repeated three times at 5 min, 10 min, 15 min, and of lactoperoxidase once at 10 min. The reaction was stopped by adding 10 ml 10 mM KI in PBS and washing three times in 10 mM Tris HCl, 150 mM NaCl, pH 7·8. For metabolic labelling with ^{35}S-methionine, 200 ml RAJI cells (grown to 5×10^5 cells/ml) were precultured in 100 ml RPMI without methionine (Seromed) containing 10% FCS for 30 min, and 1 mCi S^{35}-methionine (NEN) then added. After 16 hr of labelling, cells were washed three times in 10 mM Tris/HCl, 150 mM NaCl, pH 7·8. After labelling, cells were lysed in 1 ml 10 mM Tris/HCl, 150 mM NaCl, pH 7·8, 2 mM PMSF, 1 mM DFP 1% NP_4 for 30 min, 0°, centrifuged at 11,000 ***g*** for 10 min and stored at −80° until used.

Immunoprecipitation of membrane proteins

After centrifugation at 100,000 ***g*** for 1 hr, 200 μl of neutrophil lysate and 500 μl of tonsil interphase cell or RAJI lysate were preabsorbed twice with 500 μl of human IgG coupled to sepharose (1 mg IgG/ml sepharose) for 1 hr at 4° under constant agitation, and then incubated with 100 μl M522-Sepharose (1 mg purified M522/ml sepharose) or control sepharose (2 mg 40% NH_4SO_4 precipitate of ascites of irrelevant control antibody/ml sepharose) for 2 hr at 4° under constant agitation. The sepharose beads were then washed once in 1% NP-40, 10 mM NaH_2PO_4, 1 mMNaH_2 PO_4, 1 mM PMSF, 1 mM EDTA, 1 mM EGTA, 0·1% Na-deoxycholate, pH 7·8, once in 20% sucrose in 10 mM Tris HCl, 150 mM NaCl pH 7·8, once in 10% sucrose in 10 mM Tris HCl, 150 mM NaCl pH 7·8 containing 0·5% NP40 and again three times in the

first buffer. After washing once in sample buffer without SDS, pecipitated membrane proteins were eluted by boiling the Sepharose beads in sample buffer (50 mM Tris, 2% SDS, 5% glycerin, pH 6·8) and elecrophoresed on a 5–20% SDS polyacrylamide gel (Laemmli, 1970). After impregnation with ENHANCE® (NEN) as described by the manufacturer in the case of RAJI and tonsil cells, the dried gel was exposed to a Kodak Xromat film for 5–18 days.

Binding of ^{3}H-H to RAJI cells

Binding of tritiated (^{3}H-H) to RAJI cells in the presence or absence of unlabelled H (10 mg/ml) or M522 (1 mg/ml) was performed as described (Lambris & Ross, 1982).

RESULTS

(i) Binding of M522 to C3

In an ELISA, the binding of M522 to several complement components and fragments of C3 was assessed. Figure 1a shows that M522, but not VIM12 and OKM-1, binds to C3. The binding of M522 was weaker than that of the monoclonal antibody produced against C3 (Burger *et al.*, 1982). Its maximum binding ranged from 30% to 50% that of antibody 111 (Fig. 1a). M522 did not show significant binding to any of the other complement components tested (Fig. 1b). By producing fragments of C3 by cleavage with elastase, reduction and separation on preparative SDS-PAGE, we could localize the epitope binding to M522 on the 40,000 c-terminal fragment of the alpha-chain of C3 (Fig. 1c). The fact that M522 bound to the isolated alpha- chain, to C3c and to the 40,000 MW fragment of C3 more weakly than to whole C3 (Fig. 1b *vs* fig. 1c) is due to these fragments originating from preparative SDS-polyacrylamide gels. Treatment of C3 with 0·1% SDS reduced its binding to M522 (not shown).

(ii) Molecular weight analysis of the membrane proteins recognized by M522

As M522 showed a different pattern of rosette inhibition with neutrophils, tonsil interphase cells and B-lymphoid cells (see below), lysates of these three cell types were immunoprecipitated with M522 after metabolic (RAJI cells) and lactoperoxydase-catalysed external labelling (neutrophils, tonsil cells). Figure 2 (lane b) demonstrates that M522 pecipitates a dimer of about 165,000 and 100,000 from externally labelled neutrophils. Freezing and thawing the labelled lysate three times before immunoprecipitation only yields

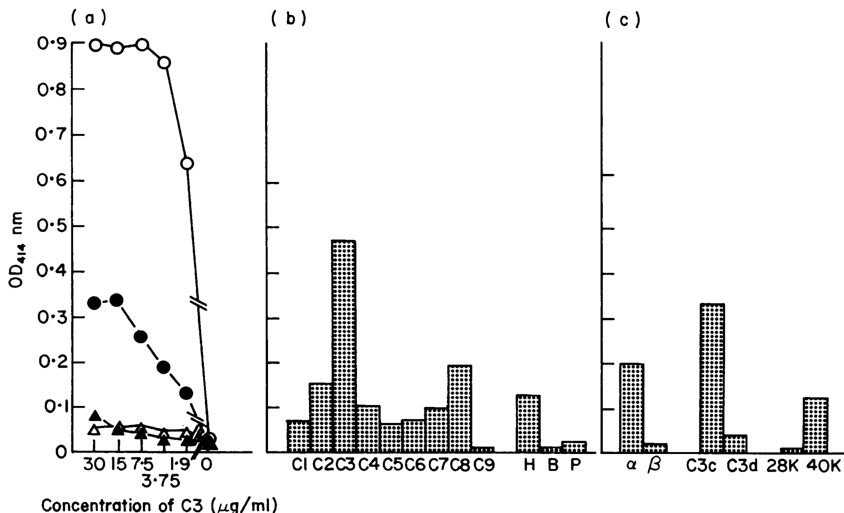

Figure 1. Binding of M522 to different complement components and fragments of C3. (a) Fifty μl of C3 in the concentrations indicated were attached to ELISA-plates and the binding of M522 (●——●), VIM12 (▲——▲), ab 111 (○——○ (all 20 μg/ml) and OKM-1 (△——△) (1:100 dilution of commercial source) assessed as detailed in Materials and Methods. (b) Different complement components at 50 μg/ml were attached to the plates and the binding of M522 (50 μg/ml) measured. (c) Ten μl C3 fragments prepared and eluted from preparative SD-gels were attached to ELISA-plates and the binding of M522 assessed. The results from one of four typical experiments are shown. Background binding (i.e. binding to BSA) averaged 0·05 (OD_{414}).

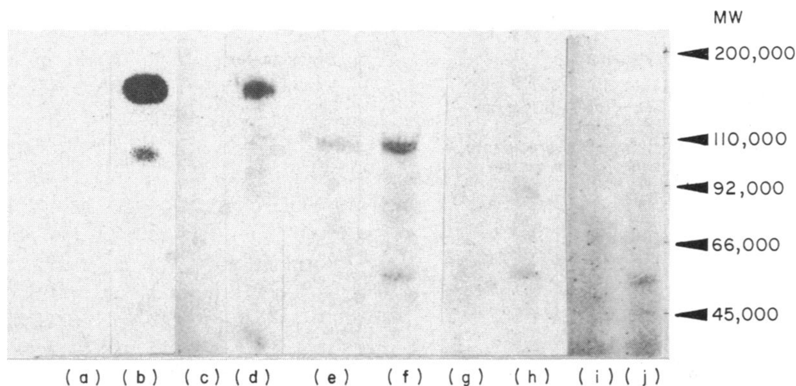

Figure 2. Molecular weight analysis of membrane proteins recognized by M522 as demonstrated on autoradiographs of 5–20% SDS-polyacrylamide gels: lanes a and b, neutrophils ^{125}I-labelled; lane c and d, neutrophils, ^{125}I-labelled, pecipitation performed after freezing and thawing the lysate three times; lanes e and f, RAJI, ^{35}S-methionine labelled; lanes g, h, i and j, tonsil interphase cells, ^{125}I-labelled (two independent experiments). lanes b, d, f, h, and j show precipitates obtained with purified M522 bound to sepharose at 1 mg/ml; lanes a, c, e. g, and i show the precipitates performed with a 40% NH_4SO_4 precipitate of a control monoclonal ascites (2 mg/ml sepharose). MW markers: myosin (200,000), β-galactosidase (110,000), phosphorylase B (92,000), BSA (66,000), ovalbumin (45,000).

the 165,000 MW band (lane d). Thus, M522 reacts with the 165,000 band which seems to be non-covalently attached to the 100,000 MW band.

On RAJI cells, however, M522 seems to react with a 50,000 MW molecule immunoprecipitated after metabolic labelling (lane f). Lanes h and j (two independent experiments) demonstrate that the same molecule could be precipitated from ^{125}I-labelled tonsil interphase cells. In one experiment (lane h), an additional 95,000 band was seen, which could, however, not be observed in all experiments (lane j). In keeping with the low amount of M522 antigen on RAJI cells, which could only be observed by a rosetting technique using M522 bound to $CrCl_3$-treated sheep erythrocytes (see Table 2) but not in immunofluorescence (Lohmeyer *et al.*, 1981), these two bands required enhancement by 'ENHANCE' and long exposure times to be visualized, especially after external labelling.

From externally labelled monocytes, M522 precipitated a similar 165,000/100,00 dimer as it did from neutrophils (data not shown).

(iii) Capacity of M522 to activate the oxydative burst of human neutrophils and monocytes

In order to ascertain whether the membrane molecule defined by M522 on neutrophils and monocytes was involved in the activation of these cells, we investigated whether $F(ab)_2$ fragments of M522 would stimulate the respiratory burst of these phagocytic cells. As shown in Fig. 3, M522-$F(ab)_2$ produced noticeable stimulation, in the case of monocytes, of similar magnitude as that produced by factor H, which we have previously shown to trigger the respiratory burst of human peripheral blood monocytes (Schopf *et al.*, 1982). Another monoclonal antibody, 669G2, reacting with human neutrophils, kindly provided by Dr Engelberger, (Mainz, W. Germany), and a 40% NH_4SO_4 precipitate of ascites from a non-producer cell line did not stimulate. The stimulation produced by M522 $F(ab)_2$ in the case of neutrophils was about 20% of that produced by zymosan and was dose-dependent, with 50 μg/ml producing optimal stimulation (data not shown).

(iv) Comparison of the capacity of M522 and OKM-1 to inhibit C3-dependent rosetting with neutrophils, tonsil B lymphocytes and B-lymphoblastoid cells

As antibodies to Mo-1 had been reported to block the rosette formation between EAC3bi and human neutrophils, we investigated the effect of M522 and two anti-Mo-1 antibodies on the binding of EAC3b, EAC3b-H, EAC3bi, and EAC3d to neutrophils, tonsil lymphocytes and the B-lymphoid cell line RAJI. In Table 1, the results obtained with M522 and OKM-1, a commercially available anti-Mo-1 monoclonal antibody are depicted. Whereas OKM-1 blocked the binding of EAC3b-H and EAC3bi to neutrophils, tonsil interphase cells and of EAC3b and EAC3b-H to

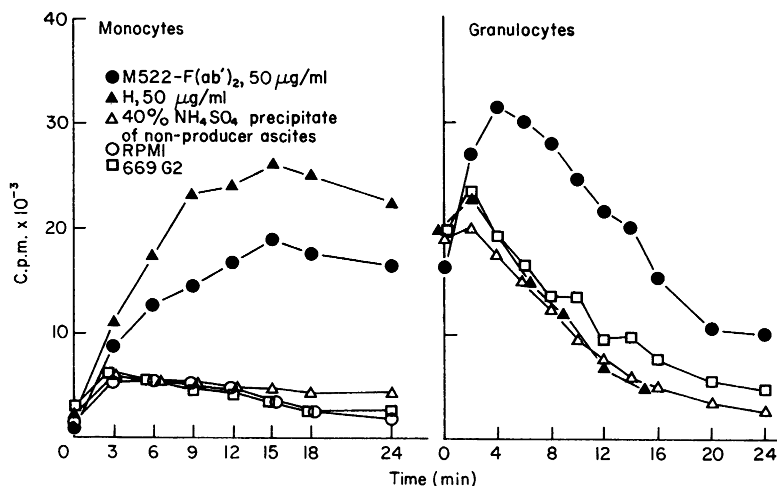

Figure 3. Effect of M522 on the respiratory burst of human monocytes and neutrophils. Chemoluminescence of monocytes and neutrophils was measured in response to stimulation with 100 μl of M522-F(ab)$_2$, 50 μg/ml (●——●), purified factor H, 50 μg/ml (▲——▲), 40% NH_4SO_4 precipitate of a control monoclonal ascites, 50 μg/ml (△——△), RPMI ○——○, the anti-neutrophil monoclonal antibody 669G2, 50 μg/ml (□——□).

Table 1. Comparison of the effects of M522 and OKM-1 on the rosette formation

	Dilution of antibody	Neutrophils		Tonsil lymphocytes		RAJI	
		M522	OKM-1	M522	OKM-1	M522	OKM-1
EAC3b	1:50	58*	54	51	26	30	30
	1:200	58	38	43	24	35	15
	1:800	50	46	40	34	41	40
	Buffer	56		35		40	
EAC3b-H	1:50	38	15	0	11	5	9
	1:200	40	17	4	12	24	14
	1:800	44	25	5	14	38	20
	Buffer	57		28		30	
EAC3bi	1:50	51	15	51	15	71	77
	1:200	51	20	38	30	70	78
	1:800	43	26	50	35	73	76
	Buffer	50		50		71	
EAC3d	1:50	ND	ND	35	32	70	80
	1:200	ND	ND	37	33	73	77
	1:800	ND	ND	48	44	68	77
	Buffer	ND		42		79	

* Values given denote % rosette formation and represent a typical experiment of two to four experiments performed. Rosette inhibition tests comparing M522 and OKM-1 were always done simultaneously. The source of M522 contained 5 mg purified antibody/ml. OKM-1 was a commercial preparation which gave good immunofluorescence with neutrophils in a 1:100 dilution.

RAJI cells, M522 did not affect rosette formation with neutrophils at all, and only blocked the binding of EAC3b-H but not of EAC3bi to tonsil interphase cells and RAJI cells. VIM-12, another anti-Mo-1 antibody, kindly donated by Dr Knapp (Vienna) behaved similarly to OKM-1 (results not shown).

(v) Inhibition by M522 of the binding of H coupled to $CrCl_3$-treated erythrocytes but not of tritiated H

In order to further analyse the inhibitory effect of M522 on rosette formation, M522 and purified factor H in the absence of C3 were coupled to $CrCl_3$-treated sheep erythrocytes. Table 2 demonstrates that E-H and E-M522 bind to RAJI cells and that their binding can be blocked by free H and M522, respectively. As an additional control, E-M522 were reacted with T lymphoblasts (JURKAT) and found to bind to 7% of these cells, which was considered as the unspecific background. In addition, free M522 is capable of reducing the binding of E-H to RAJI cells, whereas free H does not affect the binding of E-M522.

However, the binding of tritiated H to RAJI cells was not blocked by an excess of free M522. Cross-linking M522 by means of a polyclonal anti-mouse IgG also did not produce an inhibition (data not shown).

DISCUSSION

Human peripheral blood leucocytes have been shown to possess membrane-associated molecules which are antigenically related to, and in some cases possibly identical with, C4, C3, H, C6–9 and B (see introduction). On the other hand, a membrane molecule, the receptor for factor H, has been shown to be antigenically related to, but to differ in molecular weight from, serum C3 (Lambris & Ross, 1982). This molecule seems to be involved in the activation of monocytes (Schopf *et al.*, 1982) and B lymphocytes (Hammann *et al.*, 1981; Tsokos *et al.*, 1984). Here, we report observations made with a monoclonal antibody M522 which was originally produced against cells from a chronic lymphocytic leukaemia of T-cell phenotype and which was found to react with monocytes, neutrophils and 9–17% of non-adherent peripheral blood lymphocytes, including the NK-cells (Lohmeyer *et et.*, 1981). Thus, it showed a pattern of distribution similar to the Mo-1 antigen (Sanchez-Madrid *et al.*, 1983; Todd & Schlossmann, 1983).

In the first International Leucocyte Differentiation Antigen Workshop, M522 was assigned to the same cluster of monoclonal antibodies as OKM-1 (Bernard *et al.*, 1984).

Table 2. Inhibitory effect of M522 on the binding of H-coupled to $CrCl_3$-treated SRBC and of tritiated H to RAJI cells

Ligand	Inhibitor	% rosette formation (±SEM)	C.p.m. bound to 10^6 RAJI cells
E-H	BSA (1 mg/ml)	42±1·8*	
E-M522	BSA (1 mg/ml)	33±1·2	
E-BSA	BSA (1 mg/ml)	7±2·5	
E-H	H (1 mg/ml)	21±1·35	
E-M522	M522 (1 mg/ml)	12±4·0	
E-H	M522 (1 mg/ml)	29±3·3	
E-M522	H (1 mg/ml)	36±1·6	
^{3}H-H†	BSA (1 mg/ml)		618
^{3}H-H	H (10 mg/ml)		257
^{3}H-H	M522 (1 mg/ml)		666

* The results of the rosette-inhibition experiments represent the mean of four independent experiments ± SE of the mean.
† ^{3}H-H (14,000 c.p.m.) was added to 10^6 RAJI cells in the presence of BSA (1 mg/ml), H (10 mg/ml) or M522 (1 mg/ml). The results shown are from one of two similar experiments. The value obtained in the presence of unlabelled H (257 c.p.m.) represents background binding.

As shown in Fig. 2 (lane b), M522 also precipitated a dimer of about 165,000 and 100,000 MW from externally labelled neutrophils and could, therefore, react with the Mo-1 molecule.

Whether identical to Mo-1 or not, the molecule defined by M522 seems to be important for the activation of neutrophils and monocytes, since its $F(ab)_2$ fragments stimulate the respiratory burst of these cells. Our findings suggest that its 165,000 MW: chain is related to the alpha-chain of C3. It seems unlikely that these two molecules are very closely related, since $F(ab)_2$ fragments of polyclonal antibodies to serum C3 do not stain neutrophils and monocytes in immunofluorescence, nor do they precipitate anything like the M522 molecule from labelled neutrophils (T. F. Schulz, unpublished observations.) However, as C3, C4 and α_2-macroglobulin show a certain amount of sequence homology without manifesting antigenic cross-reactivity detectable by polyclonal antibodies (Tack, 1983), the cross-reactivity between C3 and the M522-membrane antigen may be indicative of a similar relationship between these two molecules. Whether the C3-like molecule previously observed on human monocytes by means of immune adherence to monkey, but not to human, erythrocytes (Burns & Cawley, 1979) could be the M522 antigen is unclear at present.

Unlike two antibodies to Mo-1, M522 did not inhibit the binding of EAC3bi to neutrophils and only blocked the rosette formation between EAC3b-H and RAJI cells or tonsil interphase cells. While this observation does not necessarily argue against the identity of Mo-1 and the membrane molecule defined by M522 on neutrophils—a monoclonal antibody to CR1 not inhibiting C3b-dependent rosette formation has been described (Hogg *et al.*, 1984)—it prompted us to investigate the relationship between the molecule recognized by M522 on B cells and the H-receptor previously reported to be antigenically related to H (Lambris & Ross, 1982). Low amounts of a molecule binding to M522 are present on B cells, as shown by both the rosette formation with $CrCl_3$-treated, M522-coupled sheep erythrocytes and the precipitation of a 50,0000 MW molecule on unreduced gels after external as well as metabolic labelling. M522 weakly inhibits the binding to RAJI cells of H coupled to $CrCl_3$-treated erythrocytes, but not of tritiated H. The H receptor has been reported to be a 100,000/50,000 dimer on unreduced, and a 50,000 monmomer on reduced, gels and to be present on RAJI cells in about $4{\cdot}2 \times 10^6$ copies/cell (Lambris *et al.*, 1980; Lambris & Ross, 1982). Therefore, in spite of the suggestive finding that both the M522 antigen and the H receptor are antigenically related to C3, these data do not prove that they are, in fact, identical. Neither do they prove the contrary, however, since a monoclonal antibody could fail to block the binding of tritiated H to its receptor if reacting with an epitope at a certain distance from the binding site for H. The fact that we could not always precipitate the 100,000 chain of the H receptor could be explained by M522 only binding to its 50,000 chain. Finally, as M522 reacted with C3 more weakly than did a monoclonal antibody produced by immunization with C3, it might also react weakly with the H-receptor and, therefore, not be the ideal reagent to precipitate or visualize the H-receptor in immunofluorescence.

Whether the antigenic relationship between the alpha-chain of C3 and a neutrophil/monocyte activation molecule extends to the H-receptor, a B-cell activation molecule, is therefore not yet clear.

Our finding that a 50,000 molecule from B lymphocytes is antigenically related to C3 raises the question as to whether the 50,000 molecule is identical to the C3-like molecule previously reported on B cells.

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