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Characterization of the C1q receptor on a human macrophage cell line, U937

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The binding of C1q to the human macrophage cell line U937 has been studied. Fluorescence microscopy with fluorescein-conjugated $F(ab')_2$ anti-C1q antibody showed that 100% of the cell population is able to bind exogenous C1q. Monomeric C1q binding to U937 cells is very weak at normal ionic strength (I0.15) and was therefore investigated at I0.07, conditions which stabilize the binding. However, aggregation of C1q on dextran sulphate or a lipid A-rich lipopolysaccharide allowed a firm, binding at I0.15. Quantitative binding studies with monomeric ^{125}I-C1q showed a concentration-dependent, saturable, specific and reversible binding involving specific membrane receptors. Scatchard plots of C1q binding indicated $[1.6 \pm 0.7$ (1 S.D.)$] \times 10^6$ sites per cell with an equilibrium constant of $(2.9 \pm 1.8) \times 10^7 M^{-1}$ at I0.07. The location of the molecule region mediating C1q binding was established with collagen-like fragments prepared by partial pepsin digestion, confirming earlier results obtained by inhibition studies.

Specific receptors for C1q have been demonstrated on monocytes, macrophages, polymorphonuclear cells, B-lymphocytes, lymphoblastoid cell lines, platelets and endothelial cells by using fluorescent, radiochemical or rosetting techniques (Tenner & Cooper, 1980, 1981). The interest in these receptors, whose biological significance was so far uncertain, increased after the finding that C1 inhibitor rapidly dissociates the $C\bar{1}r$ and $C\bar{1}s$ subunits of $C\bar{1}$ from C1q upon C1 activation in normal serum (Sim & Reboul, 1981). This dissociation process allows the fixation of C1 activators on the surface of cells expressing C1q receptors and the initiation of biological responses after receptor clustering. As detailed studies on membrane receptors are likely to require large amounts of cells, and as normal human monocytes or macrophages are difficult to obtain in large number and in pure form, we have chosen the human macrophage cell line U937 as a model to characterize its surface-membrane receptors for C1q. The U937 cell line was established in 1976 by Sundström & Nilsson from a patient with histiocytic lymphoma; it manifests, especially after activation, several features of normal human monocytes/macrophages, which might help in the study of these cells (Anderson & Abraham, 1980). We report here that the U937 cell line bears approx. 1.6×10^6 trypsin-resistant, Pronase-sensitive binding sites per cell, which specifically recognize the collagenous-like tail of the C1q molecule. The binding affinity of these cells depends on the ionic strength and on the state of aggregation of C1q.

Abbreviations used: CIRC, Centre International de Recherches sur le Cancer; Fcγ, *C*-terminal half of heavy-chain dimer from IgG (immunoglobulin G); $F(ab')_2$, *N*-terminal halves of heavy chains of Ig joined by inter-heavy-chain disulphide bond to light chains; Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid; SDS, sodium dodecyl sulphate; the nomenclature of the complement components and subcomponents is that recommended by the World Health Organisation (1968); activation of a component is indicated by an overbar.

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Materials and methods

Cell line

U937 cells obtained from Dr. G. Lenoir of the CIRC, Lyon, France, were maintained in suspension culture at concentrations of about 1×10^6/ml

in RPMI 1640 medium supplemented with 10% (v/v) foetal-calf serum, Hepes (15mM), glutamine (2mM), penicillin (100units/ml) and streptomycin (100μg/ml), all from Flow Laboratories, Irvine, Ayrshire, Scotland, U.K. Before the experiments, cells were washed twice, resuspended in RPMI alone and the viability (always more than 90%) was assayed by ethidium bromide exclusion. U937 cells were activated by culturing them for 24–48h in the above medium supplemented with 5–10% (v/v) supernatants from human mixed lymphocyte cultures obtained on day 6 of culture. Activation was assessed from the marked morphological changes, as reported by Koren *et al.* (1979) and the enhanced ingestion of non-opsonized yeasts (Shaala *et al.*, 1979).

Purification of C1q

C1q was isolated from fresh frozen human plasma by differential elution of $C\bar{1}$ subcomponents bound to insoluble IgG–ovalbumin aggregates and further purified by CM-cellulose chromatography (Arlaud *et al.*, 1979).

Purified C1q was stored at −80°C in 0.23M-acetate/0.3M-NaCl buffer, pH5.2.

Enzymic digestion of C1q

C1q was digested with pepsin by the method of Reid (1976). Briefly, 1mg of unlabelled C1q dialysed against 100mM-sodium acetate buffer, pH4.45, was incubated with 33μg of pepsin (Sigma) at 37°C for 20h. After centrifugation, the supernatant was adjusted to pH8.0 with Tris powder and applied to a Sephacryl S-300 column equilibrated with 50mM-Tris/0.15M-NaCl/1mM-EDTA buffer, pH7.4, and run at 4°C. Elution was carried out with the same buffer. The first peak, eluted near the void volume of the column, was pooled, adsorbed on insoluble immune complexes in order to remove any incompletely digested C1q molecules, then concentrated and stored at 4°C.

Collagen-like fragments were partially digested with collagenase as described by Reid (1976) for oxidized B-chains of C1q.

Briefly, 1mg of collagen-like fragments, dialysed against 50mM-Tris/HCl/5mM-$CaCl_2$/0.25mM-*N*-ethylmaleimide, pH7.4, was incubated wtih 30μg of collagenase at 37°C for 20h. After removal of any precipitate by centrifugation, the resulting supernatant was applied to a Sephacryl S-300 column equilibrated with the same buffer as above. The first peak, eluted near the void volume of the column, was pooled, concentrated and stored at 4°C. A limited collagenase digestion was performed under the same conditions but in the presence of 10mM-EDTA. All three preparations of collagen-like fragments were examined by SDS/polyacrylamide-gel electrophoresis (Fairbanks *et al.*, 1971). Their ability to reconstitute C1 in the presence of C1r and C1s was evaluated by sucrose-density-gradient centrifugation (Martin & Ames, 1961) and also by the activation of proenzymic C1s, as judged from SDS/polyacrylamide-gel electrophoresis (Villiers *et al.*, 1982).

Radiolabelling of C1 and collagen-like fragments

Lactoperoxidase-catalysed iodination was performed by a modification of the method of Heusser (1973) to a specific radioactivity of about 0.1μCi/μg. Briefly, 500μg of C1q or collagen-like fragments, 250μCi of carrier-free ^{125}I (100mCi/ml; CEA, Saclay, France), 0.03μg of NaI, 5μg of lactoperoxidase and 5μl of 0.003% H_2O_2 were incubated at 0°C for 15min in a total volume of 350μl; 60μg of NaI and 0.3μg of NaN_3 were then added to terminate the reaction.

The mixture was dialysed against three changes of phosphate-buffered saline (145mM-NaCl/10mM-sodium phosphate, pH7.2) and the resulting iodinated proteins, stored at 4°C, were spun at 40000*g* for 90min just before use to remove any aggregate.

In supernatants, proteins were found to be monomeric when judged by sucrose-density-gradient centrifugation.

Formation of C1 with radiolabelled C1q

C1 was reconstituted by incubating purified radiolabelled C1q with a 50-fold molar excess of proenzymic C1r and C1s (Arlaud *et al.*, 1980) in 5mM-$CaCl_2$/5mM-triethanolamine/145mM-NaCl, pH7.4, for 30min at 0°C.

C1 was used without further separation from unchanged C1r and C1s.

Fluorescence microscopy

To detect C1q binding, cells were incubated with C1q (10μg/10^6 cells) or buffer for 30min on ice in RPMI medium plus the required amount of 5% (w/v) dextrose to achieve the desired ionic strength (*I*0.07). The cells were then washed thrice with the same medium before adding an appropriate dilution of fluorescein-conjugated $F(ab')_2$ anti-C1q at the same ionic strength. After 30min on ice, the cells were washed thrice with the above buffer in the cold. Wet mounts of the cells were examined on a Zeiss standard microscope by both contrast and fluorescence epi-illumination microscopy.

Binding assay

Immediately before use, washed U937 cells were centrifuged to a pellet of $(1–4) \times 10^6$ cells in 1.4ml conical polypropylene microcentrifuge tubes. The

cell pellet was then incubated with various amounts of labelled C1q or its fragments (0.5–50 μg) in RPMI/0.02M-Hepes (pH 7.2)/0.5% ovalbumin plus the required amount of 5% dextrose/0.02M-Hepes (pH 7.2)/0.5% ovalbumin to achieve the desired ionic strength in a final volume of 200 μl. After incubation for 1 h at 37°C or 3 h at 0°C (unless otherwise specified) with periodic agitation, triplicate 50 μl aliquots were each layered on 300 μl of Percoll (Pharmacia) diluted in phosphate-buffered saline (1:4, v/v; $d = 1.035$) in polypropylene microcentrifuge tubes. The tubes were spun for 1 min in a Beckman microcentrifuge and the tips containing the cell pellet were cut off and counted separately to measure the total bound radioactivity. The centrifugation time (1 min) is short compared with the dissociation time of bound C1q (about 45–60 min, after washing and adding new medium). Thus it was not necessary to correct for dissociation of bound C1q during centrifugation.

The concentration of free labelled protein was calculated from the radioactivity in 20 μl aliquots of supernatants obtained by centrifuging the residual amount of incubation medium.

A correction for non-specific binding was applied at each ligand concentration by subtracting from the experimental values the amount of radioactivity sedimenting through Percoll when cells had been either omitted or incubated with labelled ligand in the presence of a large (100-fold or greater) excess of unlabelled ligand.

Non-specific uptake measured by one of these two methods, which give equivalent results, was 5–25% of the specific radioactivity bound to cells.

The number of molecules of ligand bound per cell, r, was calculated by the relationship:

$$r = \frac{6.02 \times 10^{20}}{n \cdot \mathrm{SpA}} \text{ Cor. Exp.}$$

where SpA is the specific radioactivity in c.p.m./mmol of the ligand, n is the number of cells applied to each microcentrifuge tube, and Cor. Exp. is the average c.p.m. counted in cell pellets after correction for non-specific binding.

In the present paper the molar concentration of all proteins or fragments is based on M_r 4.6×10^5 for C1q (Reid, 1983), 1.79×10^5 for peptic fragments (Sasaki & Yonemasu, 1983), 1.23×10^5 for collagenase fragments and 1.45×10^5 for collagenase fragments obtained in the presence of EDTA.

IgG, fibronectin and polyanions

Human IgG myeloma proteins of four subclasses were purified from serum by precipitation with 50%-satd. $(NH_4)_2SO_4$, followed by chromatography on a DEAE-cellulose column (Fine & Steinbuch, 1970).

Purified human fibronectin was obtained by precipitation in the cold with heparin (Stathakis & Mosesson, 1977).

The following polyanions were used: dextran sulphate (M_r 15000) was purchased from Sochibo, Boulogne-sur-Seine 92100, France; lipopolysaccharide, from *Salmonella minnesota* Re 595, (M_r 3100) was from Sigma; tRNA (M_r 25000–30000), poly(L-glutamic acid) (sodium salt; M_r 70000) and poly(5'-adenylic acid) (type I, potassium salt, $M_r > 10000$) were all from Sigma.

Protein determination

Protein concentrations were calculated from A_{280} by using $A^{1\%}_{1cm} = 13.8$ for IgG, 6.82 for C1q and 2.1 for peptic fragments (Siegel & Schumaker, 1983) assuming also the same value for collagenase fragments.

Results

Immunofluorescence staining

None of the cells was labelled by fluorescein-conjugated $F(ab')_2$ anti-C1q in the absence of a previous exposure to purified exogenous C1q. Approx. 100% of the cells showed C1q on their surface after incubation with purified exogenous C1q for 30 min at 0°C, followed by washing, as described in the Materials and methods section. The membrane immunofluorescence staining, although detectable at normal ionic strength (I0.15) was much more intense, with large patches of fluorescence at low ionic strength (I0.07); a few cappings were observed at 37°C. Subsequent experiments, unless otherwise noted, were performed at I0.07.

To rule out pinocytosis, U937 cells were comparably examined for C1q binding in the presence or absence of 2mM-NaN_3; no change in the staining was observed.

Kinetics of association and dissociation of C1q and U937 cells

Rates of association were dependent on both the concentration of C1q and the temperature at which the cells were incubated with labelled C1q. Fig. 1(*a*) shows the time course of association of ^{125}I-C1q with cells at 0°C and 37°C. In these experiments, C1q was used at a low concentration (7 μg/ml) to allow easier analysis of the phenomenon. Binding reached its maximum level after 1 h at 37°C and after 3 h at 0°C. At 10-fold higher C1q concentrations the reaction was considerably faster, with binding equilibrium achieved within 10 min at 0°C (result not shown).

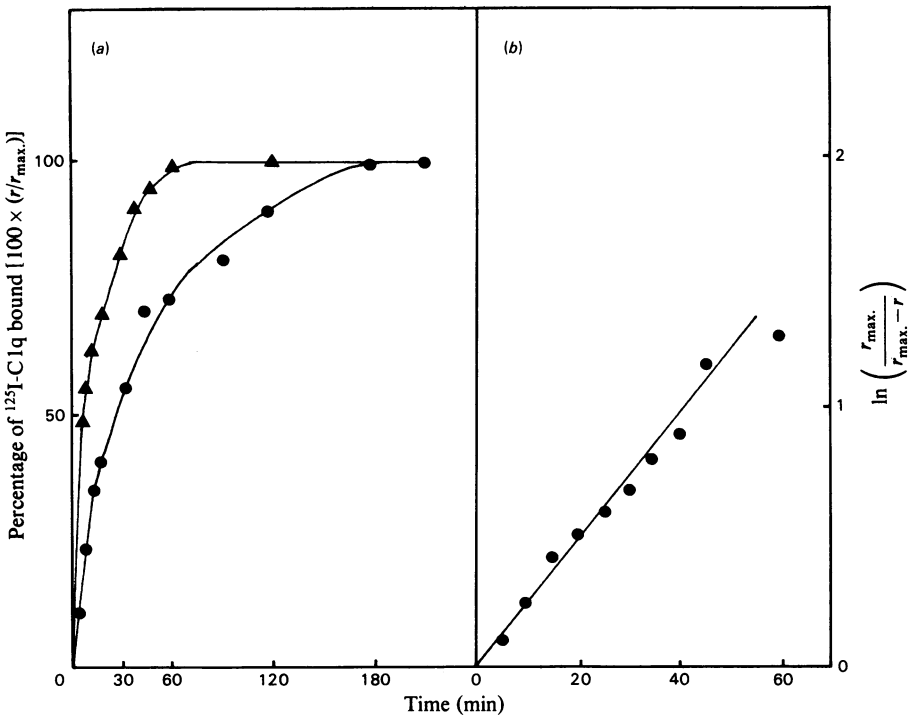

Fig. 1. *Binding of C1q to U937 cells*

(*a*) Time course of binding. U937 cells (1×10^7/ml) were incubated at 0°C (●) or 37°C (▲) with 7 μg of ^{125}I-C1q/ml. At the indicated times, samples were removed from the incubation vessels and bound ligand was separated from free. Data were corrected for non-specific binding. The experiment was repeated twice with similar results. (*b*) Plot of data, given in (*a*) at 4°C, according to a first-order reaction. The value for $r_{max.}$ was the average number of molecules of ligand bound per cell at equilibrium and r was the same parameter at earlier time points.

The reaction followed pseudo-first-order kinetics (Fig. 1*b*).

Next, we decided to find out whether the binding reaction was reversible by performing the following experiment at 37°C. U937 cells that were at equilibrium with sub-saturating amounts of ^{125}I-C1q at *I*0.07 were divided into four portions. Two portions received a large excess of unlabelled ligand, either at *I*0.15 or at *I*0.07.

In the two others, unbound ^{125}I-C1q was washed away and replaced by fresh medium at normal or low ionic strength. Periodically, bound ligand was separated from the free material.

In the presence of excess ligand at *I*0.15, virtually all (97%) of the cell-bound ^{125}I-C1q was dissociated within 30min. Dissociation obtained by washing and resuspension in fresh medium proceeded more slowly, specially at low ionic strength, but could go to completion.

Cells dissociated from C1q were able to rebind the same amount of fresh ^{125}I-C1q, suggesting that loss of ^{125}I-C1q from the cells was a true ligand–receptor-dissociation phenomenon and was not shedding of receptor–ligand complexes.

Affinity of binding and total number of receptor sites per cell

The binding of purified monomeric ^{125}I-C1q to U937 cells was dose-dependent and saturable. Fig. 2 shows a typical binding curve. Scatchard plots of the data obtained with labelled C1q and collagen-like fragments are shown in Fig. 3, and binding parameters are listed in Table 1.

Comparison of binding parameters at 0°C and 37°C on the one hand and between activated and non-activated cells on the other hand exhibited no significant difference.

Specificity of the C1q-binding site

The specificity of the C1q-binding site of U937 cells was studied by incubating cells and a small amount of ^{125}I-C1q (1 μg/ml) with or without various concentrations of unlabelled competing proteins, namely C1q, fibronectin and IgG1 (Fig. 4). After 2h of incubation, bound ligand was separated from the free material and the amount of ^{125}I-C1q bound was plotted against the competitor-protein concentrations.

Table 1. *Parameters of binding of C1q and collagen-like fragments to U937 cells*

Protein	$10^{-6} \times n$ (molecules/cell)	K_a ($\times 10^{-6}$ M^{-1})	r*	No. of expts.
C1q	1.6±0.7†	29±18†	0.92±0.05†	9
Peptic fragments	1.4±0.9	1.8±0.6	0.94±0.07	7
Collagenase/EDTA fragments	1.2±0.6	1.4±0.5	0.95±0.07	3
Collagenase fragments	0.15	4.2	0.94	2

* r is the linear correlation coefficient (absolute value) derived from least-squares analysis of Scatchard plots of binding.
† Data represent means±S.D.

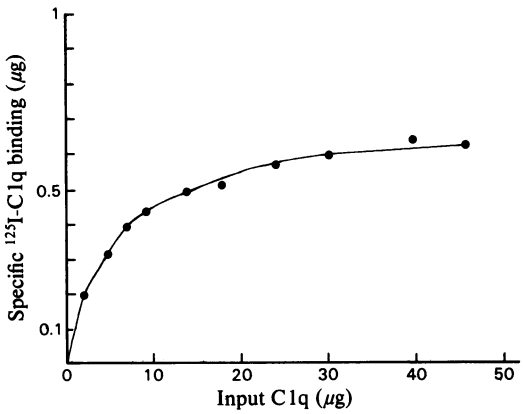

Fig. 2. *C1q binding as a function of C1q concentration*
Cells (2.5×10^6/ml) were incubated for 2h at 37°C with increasing concentrations of ^{125}I-C1q at *I*0.07 in a total volume of 200μl. Results have been corrected for non-specific binding as described in the Materials and methods section.

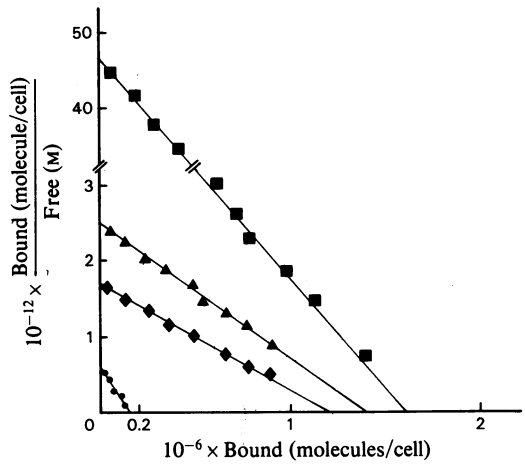

Fig. 3. *Scatchard analysis of the binding of C1q and collagen-like fragments to U937 cells*
Cells (1×10^7/ml) were incubated at *I*0.07 with various concentrations of C1q (2–43μg/ml; ■) or collagen-like fragments (6–100μg/ml) obtained with pepsin (▲), collagenase (●) or collagenase in the presence of EDTA (◆). After 2h at 37°C, bound ligand was separated from free and non-specific binding was subtracted. Continuous lines are the least-squares fits whose slope estimates the association constant and the *x*-axis intercept gives number of binding sites per cell.

C1q binding was specific, since simultaneous addition of a 50–500-fold molar excess of unlabelled purified C1q blocked the uptake of the radiolabelled protein.

The inhibition equilibrium constant, K_i, estimated for unlabelled C1q by the reciprocal of the molar concentration required for 50% inhibition of ^{125}I-C1q binding was 1.2×10^6 M^{-1}.

This value was about the same as the K_a value measured for ^{125}I-peptic fragments in the direct binding assay and was 24-fold less than the K_a value assayed in the same way for ^{125}I-C1q.

To determine whether the marked inhibitory effect of fibronectin on C1q binding shown in Fig. 4 was reflecting interactions between fibronectin and the collagenous region of C1q molecule or mutual interference in the binding of the two proteins to the same receptor, we first incubated U937 cells with fibronectin for 30min at 37°C before washing and adding ^{125}I-C1q. Under these conditions no relevant decrease in C1q binding was found. From the 50% inhibitor dose, a K_i of 4×10^6 M^{-1} could be estimated for the binding of fibronectin to C1q.

Large amounts of monomeric IgG1 (20–200μg/ml) did not inhibit ^{125}I-C1q binding, indicating the lack of relationship between C1q receptors and Fc receptors (Fig. 4).

Effect of iodination on C1q binding

To investigate the possibility that iodination resulted in denaturation of radiolabelled C1q and produced effects other than 'artefactually' enhanced affinity of binding, the following control experiment was performed.

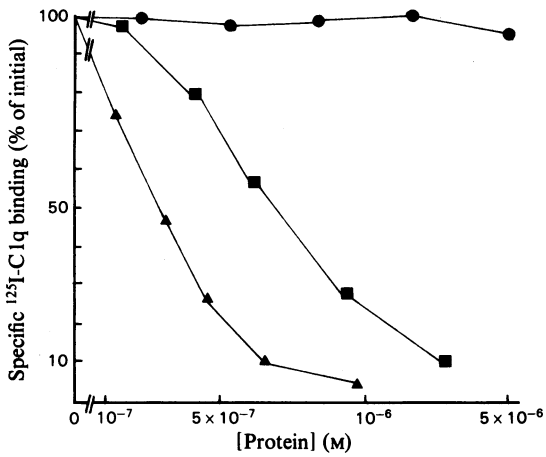

Fig. 4. *Specificity of ^{125}I-C1q binding to U937 cells* Cells (1×10^7/ml) were incubated with 1 μg of radiolabelled C1q/ml plus increasing concentrations of unlabelled competing proteins, either C1q (■), fibronectin (▲) or monomeric IgG1 (●), for 2h at 37°C at *I*0.07. Binding was assayed as described in the Materials and methods section.

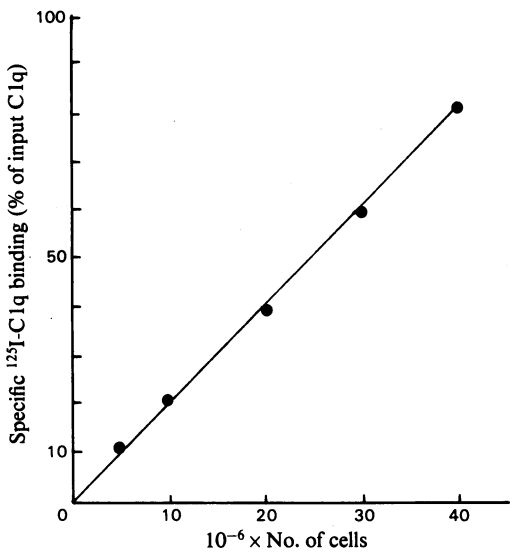

Fig. 5. *Effect of iodination on C1q binding to U937 cells* A low concentration of ^{125}I-C1q (1.6μg/ml) was incubated with increasing numbers of U937 cells at *I*0.07. Binding was assayed as described in the Materials and methods section.

A small amount (1.6μg/ml) of ^{125}I-C1q was incubated at 37°C for 2h with increasing numbers of U937 cells. It can be seen from Fig. 5 that binding is proportional to the number of cells added, and at least 82% of the C1q input is capable of being bound. By extrapolating the curve of Fig. 5 we might expect that most of the radioactive material would be bound if enough cells could be added.

Location of the region of the C1q molecule involved in the binding to U937 cells

Three lines of evidence were consistent with the fact that C1q binds to cell receptors via its collagen-like region. Involvement of the collagen part of C1q in the binding to the U937 cell receptor was revealed by earlier experiments with purified fragments (Table 1) or competition with fibronectin (Fig. 4). Another experiment was designed to study the relative ability of U937 cells to bind free C1q and C1. Equivalent amounts of ^{125}I-C1q in free form or in complex with C1r2–C1s2 were incubated with the cells. No specific binding was detected with reconstituted C1, whereas binding was entirely restored in the presence of 10mM-EDTA.

Effect of ionic strength and C1q aggregation on binding to U937 cells

The effect of ionic strength on the ability of U937 cells to bind monomeric ^{125}I-C1q or peptic fragments is shown in Fig. 6. Ligand binding proportionally decreased as the ionic strength was increased from 0.05 to 0.15. At normal ionic strength (*I*0.15), 14-fold less binding than that detected at *I*0.05 was observed with C1q and 4-fold less with peptic fragments. In order to enhance C1q binding at physiological ionic strength, we used the ability of different polyanions to interact with C1q and to present it as a cluster on their surface. In these experiments, cells were incubated at normal or low ionic strength, together with a constant concentration of ^{125}I-C1q and various amounts of the following compounds: dextran sulphate, a lipid A-rich lipopolysaccharide derived from the Re 595 mutant strain of *Salmonella minnesota*, tRNA, poly-(L-glutamic acid) and polyadenylic acid. No enhancement of C1q binding at *I*0.15 was observed with the last three polyanions.

In contrast, C1q binding at *I*0.15 was increased in the presence of dextran sulphate or lipopolysaccharide by 5–6-fold and 3-fold respectively (Fig. 7). It is interesting to note, however, that dextran sulphate markedly reduced C1q binding at *I*0.07. Control experiments eliminated a direct interaction between polyanion and the cell surface; no enhancing effect at *I*0.15 was found when the two polyanions were first incubated with cells for 30min at 37°C, before washing and adding C1q. Also, when peptic fragments were used, instead of intact C1q molecule, no enhancement of the binding at *I*0.15 was observed with the polyanions. Our

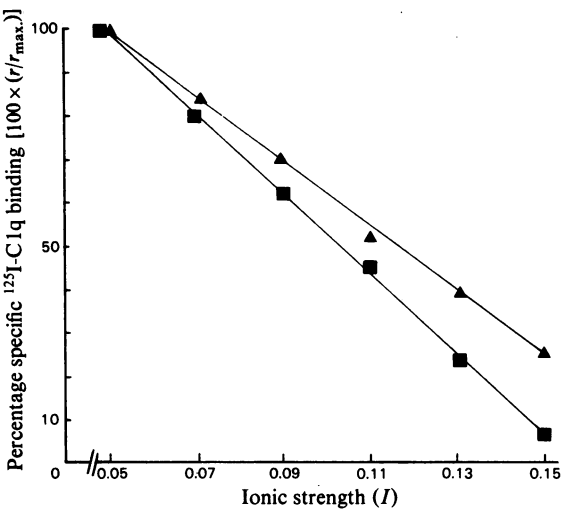

Fig. 6. *Monomeric C1q or collagen-like fragments binding as a function of ionic strength*
U937 cells (1×10^7/ml) were incubated with 7 μg of C1q/ml (■) or 12 μg of peptic fragments/ml (▲) at various ionic strengths. After 2h at 37°C, binding was assayed as described in the Materials and methods section. The value for $r_{max.}$ was the maximum number of molecules bound per cell at the lowest ionic strength used (i.e. *I*0.05) and *r* was the same parameter at different (higher) ionic strengths.

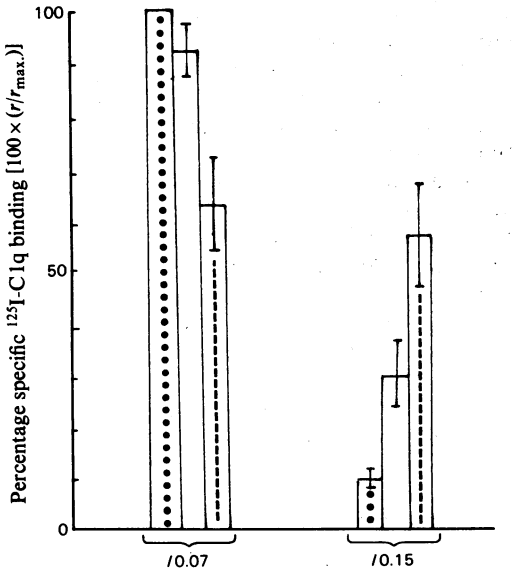

Fig. 7. *Effect of aggregation on binding of C1q to U937 cells at normal (I0.15) and low (I0.07) ionic strength*
Cells (1×10^7/ml) were incubated for 2h at 37°C with 16 μg/ml ^{125}I-C1q alone (▣) or mixed with various amounts of two polyanions: LPS from *Salmonella minnesota* Re 595 (□) or dextran sulphate (▥). The optimal polyanion/C1q ratio chosen, enhancing C1q binding at normal ionic strength, was 1 : 1 (w/w). $r_{max.}$ refers to the maximum number of C1q molecules bound per cell at *I*0.07 with C1q alone.

results thus support the earlier proposal (Gabay *et al.*, 1979) that C1q does bind to cell receptors at normal ionic strength when presented as aggregates on multivalent C1 activators.

Mild proteolytic digestion of U937 cells

The membrane receptors for C1q on U937 cells (10^7/ml) were resistant to trypsin (1 mg/ml) or collagenase (2 mg/ml) digestion for 20 min at 37°C. Longer incubations or higher concentrations of trypsin produced significant loss of viability and destruction of cells. In contrast, C1q receptors on U937 cells (10^7/ml) were decreased from $[1.6 \pm 0.7$ (1 S.D.)$] \times 10^6$ to $(0.7 \pm 0.4) \times 10^6$ sites per cell after 25 min incubation at 37°C with 2 mg of Pronase/ml. The association constant of the residual sites was unchanged.

C1q receptors returned to control levels within 48h after Pronase treatment. During this 48h culture period, the cells proliferated and increased in number 3-fold. The ability of bound C1q to protect receptor sites from Pronase digestion was studied in the following experiment. U937 cells (2×10^7/ml) were first incubated for 1h at 37°C with buffer or a high concentration of C1q (140 μg/ml) at *I*0.07 to saturate the binding sites. Cells were rapidly washed free of unbound C1q and incubated with Pronase under the conditions described. Then bound C1q was completely dissociated by washing extensively in a normal-ionic-strength medium and a binding assay was performed with a lower amount of ^{125}I-C1q. Pronase digestion of C1q receptors was not prevented by bound C1q and again caused a 55% decrease in binding of C1q.

Discussion

The data presented here indicate that the human macrophage cell line U937 is able to bind purified exogenous C1q. Quantitative binding studies using monomeric ^{125}I-C1q showed a concentration-dependent, saturable, specific and reversible binding, implying the presence of specific membrane receptors. The U937 cells contain either a single type of receptors for C1q or several receptors with similar affinities as supported by the linearity of Scatchard plots of the binding data (Fig. 3). The Scatchard plots of C1q binding indicated also 1.6 ± 0.7 (1 S.D.) sites per cell which is an average

value and does not preclude any possible inhomogeneity in the cell population. It is unlikely that any possible small amount of pinocytosis significantly affects the binding parameters, since studies carried out at 37°C give similar results to those obtained in studies performed at 4°C, at temperature at which interiorization is not expected to occur. Rates of association of C1q with U937 cells were far slower than those reported by Tenner & Cooper (1980) with blood mononuclear cells. This difference may be accounted for by the higher C1q concentrations used by those authors.

Using membrane immunofluorescent staining after repeated washings, Loos *et al.* (1980, 1981) have revealed the presence of C1q on the surface of human peritoneal and alveolar macrophages. They hypothesized that C1q synthesized by macrophages might have an Fc-receptor-like function on the membrane during the secretion phase. By the same technique, we were unable to detect any C1q on the surface of U937 cells, even after activation. This observation can be compared with results reported by Minta & Pambrun (1983), who failed to detect C1q in the culture supernates of U937 by functional tests; those authors found, however, that 20–40% of the cells were positive for C1q in a haemolytic plaque assay. Thus our negative results might be explained by the low C1q synthesis and the negligible binding of C1q at normal ionic strength.

U937 cells have previously been shown to have many properties of normal human monocytes, including similar binding parameters for subclasses of human myeloma IgG (Anderson & Abraham, 1980; Alexander *et al.*, 1978). Activation of U937 cells or normal human macrophages in response to conditioned medium from mixed lymphocyte culture has been found to result in a dose- and time-dependent increase (up to 10-fold) in the surface density of Fcγ receptor sites (Guyre *et al.*, 1981). However, the average number of C1q receptors per cell was not significantly different between non-activated and activated U937 cells.

The C1q receptors on lymphocytes (Sobel & Bokisch, 1975) and lymphoblastoid cell lines (Ghebrehiwet & Müller-Eberhard, 1978) have been reported to be destroyed by tryptic pretreatment of the cells. As Fcγ receptors, C1q receptors on U937 cells were trypsin-resistant and partly Pronase-sensitive. Further explanation will have to await more detailed studies of the molecular nature of these receptors. C1q receptor sites returned to 100% of the control value within 48h after Pronase treatment.

The C1q receptors on U937 cells are clearly specific for the collagenous region of the C1q molecule, which contains the attachment sites for C1r and C1s and interacts with fibronectin (Isliker *et al.*, 1982). This conclusion stems from the following observations. First, as compared with the intact C1q molecule, the collagen-like fragments prepared by partial pepsin digestion bound to a similar number of binding sites. Furthermore, collagenase treatment of these fragments dramatically reduced their binding to U937 cells (Table 1). Secondly, C1q binding to cells was markedly inhibited by soluble fibronectin (Fig. 4), in contrast with data obtained by Tenner & Cooper (1980) using only a 7-fold molar excess of fibronectin over C1q. Thirdly, although free C1q was readily bound to U937 cells, no specific binding was detected with the equivalent amount of C1q in complex with C1r2–C1s2.

The present study clearly shows that the affinities of U937 cells for monomeric C1q, and to a less extent for peptic fragments, were markedly increased with reducing ionic strength below I0.15 (Fig. 6); this ionic-strength-dependence has already been reported (Sobel & Bokisch, 1975; Tenner & Cooper, 1980). With the aim of presenting C1q as a cluster on a surface, we took advantage of earlier experiments demonstrating that purified C1q interacts directly with different polyanions known to activate C1 (Van Schravendijk & Dwek, 1982). Among the five polyanions tested, two of them (i.e. dextran sulphate and a lipid A-rich lipopolysaccharide) allowed a firm multisite binding at normal ionic strength. This point emphasizes the potential physiological importance of C1q receptor: it might mediate typical biological functions of monocytes/macrophages such as phagocytosis or cytotoxicity.

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