Surfactant protein D binding to alveolar macrophages

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Surfactant protein D (SP-D) is a lung-specific protein, synthesized and secreted by lung epithelial cells. It belongs to group III of the family of C-type lectins; each member of this group has an unusual overall structure consisting of multiple globular 'head' regions (which contain the C-type lectin domains) linked by triplehelical, collagen-like, strands. This group includes the surfactant protein A (SP-A) and the serum proteins mannan-binding protein, conglutinin and collectin-43, all of which have been shown to bind to the C1q receptor found on a wide variety of cells, including macrophages. Both SP-D and SP-A have been shown to enhance oxygen radical production by alveolar macrophages. Although this strongly suggests a direct interaction between SP-D and a specific receptor on alveolar macrophages, it is still unclear whether SP-D binds to the same receptor used by SP-A and/or C1q. Human SP-D was isolated from amniotic fluid and was radiolabelled using ^{125}I. Alveolar macrophages were isolated from human bronchioalveolar lavage fluid, and also from bovine lung washings, by differential adhesion to 24-well tissue-culture plates. The study was carried out using EDTA-containing buffers, to eliminate Ca^{2+}-dependent C-type lectin binding, and was also carried out at 4 °C to eliminate possible internalization by the cells. ^{125}I-SP-D showed specific binding to alveolar macrophages in both a time- and concentration-saturable manner. The binding was inhibited, by approx. 90%, on addition of a 200-fold excess of unlabelled SP-D. The apparent dissociation constant (K_d) was $(3.6 \pm 1.3) \times 10^{-11}$ M, based on the assumption that native SP-D is assembled as a dodecamer of 12 identical polypeptides of 43 kDa to yield a protein of 516 kDa. C1q was also shown to bind alveolar macrophages (K_d 3×10^{-6} M), but addition of C1q did not show inhibition of the binding of ^{125}I-SP-D to the macrophages. We conclude that SP-D binds specifically to alveolar macrophages and the receptor involved is different from that utilized by C1q.

INTRODUCTION

Surfactant protein D (SP-D) is a collagenous glycoprotein which is synthesized and secreted into the pulmonary airspaces by lung epithelial cells (Persson et al., 1988, 1989, 1990). SP-D has an unusual overall structure, i.e. N-terminal collagen-like domains connected to C-terminal globular domains which display C-type lectin activity (Rust et al., 1991). This type of structure is found in another surfactant protein, SP-A, and also in serum proteins such as collectin-43 (CL-43), conglutinin and mannan-binding protein (MBP), which comprise a subfamily (group III) of the C-type lectins (Drickamer, 1988; Thiel and Reid, 1989; Rust et al., 1991; Holmoskov et al., 1993; Lu et al., 1993). C1q, a complement protein, also has a similar structure (Reid and Porter, 1976); however, it exhibits an affinity, probably via ionic interactions, for a very wide range of substances, such as IgG- and IgM-containing immunocomplexes, lipopolysaccharides and ligand-bound C-reactive protein, rather than displaying carbohydrate-binding properties (Reid, 1989; Sim and Reid, 1991).

SP-A is considered to play a variety of biological roles within the surfactant, for example it has been shown to bind specifically to alveolar type-II cells and cause inhibition of the secretion of [^{3}H]phosphatidylcholine, a marker of surfactant phospholipids (Kuroki et al., 1988; Wright et al., 1989). There are controversial reports about the precise binding site on the SP-A molecule, which is responsible for its interaction with cell-surface receptors. Murata et al. (1993) have suggested that the non-collagenous, C-terminal lectin domains of SP-A are responsible for the binding, as judged by studies employing collagenase digestion of SP-A and use of a monoclonal antibody which recognizes the C-terminal part of the collagenase-resistant fragments. On the other hand, Tenner et al. (1989) have reported that C1q has a weak inhibitory effect on the SP-A binding to the type-II cells, thus implicating the collagen-like regions.

SP-A has been reported to enhance the uptake of particles by alveolar macrophages and to potentiate antibacterial functions (van Iwaarden et al., 1990, 1992b). This binding of SP-A to alveolar macrophages was inhibited by addition of C1q or type-V collagen (Pison et al., 1992), which again implicates the collagen-like region of SP-A as the site of interaction for cell-surface receptors. Only a few reports have been made, so far, regarding SP-D function. Unlike SP-A, SP-D failed to inhibit the secretion of surfactant phospholipids from alveolar type-II cells (Kuroki et al., 1991a). SP-D, however, has been reported to stimulate the production of free radicals in alveolar macrophages (van Iwaarden et al., 1992a). Although it is unclear whether this phenomenon was mediated by the same mechanism(s) as those of SP-A and/or C1q, it strongly suggests that there is a specific interaction between SP-D and alveolar macrophages.

The purpose of the present study was to determine whether SP-D binds specifically to the surface of the monolayers of alveolar macrophages.

MATERIALS AND METHODS

Isolation of human SP-D

SP-D was purified according to the method of Lu et al. (1992). Crude SP-D was isolated from a pool of human amniotic fluid using two steps of lectin-affinity chromatography (maltose–Sepharose). The fraction, which was eluted using a maltose-containing buffer, was further purified by gel filtration on Superose-6 in the presence of 10 mM EDTA.

Abbreviations used: SP-D and SP-A, surfactant proteins D and A; CL-43, collectin-43; MBP, mannan-binding protein.

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Radiolabelling of SP-D

SP-D (30 μg), in 300 μl of 10 mM PBS (pH 7.4), containing 0.05% (v/v) Tween-20 and 10 mM EDTA, was iodinated with 0.5 mCi of $Na^{125}I$ by a procedure utilizing iodogen (Fraker and Speck, 1978). ^{125}I-SP-D was separated from free iodine using a PD-10 column equilibrated with 50 mM Tris-buffered saline (pH 7.4) containing 0.05% (v/v) Tween-20 and 10 mM EDTA. After addition of Ca^{2+}, the labelled protein was subjected to lectin affinity chromatography on maltose–Sepharose, and was eluted using buffer containing 10 mM EDTA. The ^{125}I-SP-D was used for the binding studies within 6 h of iodination.

Isolation of alveolar macrophages

Alveolar macrophages were initially isolated from human bronchioalveolar lavage fluid. However, to overcome the difficulty of obtaining a regular supply of cells, bovine alveolar macrophages were used in the majority of the experiments. The cell pellet obtained by centrifugation (500 *g*, 10 min at 4 °C) of the lung washings was washed three times, by similar centrifugation steps, and then resuspended in RPMI-1640 medium containing 1 mg/ml BSA (RPMI–BSA). The cell suspensions were plated, in monolayers, on to 24-well tissue-culture plates ($1.0–2.0 \times 10^6$ cells/well) for 2 h at 37 °C in 95% O_2/5% CO_2. The plates were precoated with 0.05% (w/v) gelatin to eliminate non-specific binding. Non-adherent cells were removed by washing the wells using RPMI–BSA. The cell monolayer was maintained in the same medium supplemented with 100 i.u./ml penicillin, 100 μg/ml streptomycin and 100 μg/ml kanamycin until needed. The final plating efficiency varied between 10 and 20%. The cell purity was > 90% as assessed by routine morphological examination.

^{125}I-SP-D binding study

All experiments were carried out in the buffer containing 5 mM EDTA at 4 °C, in order to eliminate lectin binding and possible internalization of the bound radiolabel. There is no evidence to suggest that SP-D needs Ca^{2+} to retain its structural integrity. Monolayers of alveolar macrophages were cultured for up to 24 h after isolation and then the culture plates were placed on ice, and then the medium was removed and each well was washed three times (1 ml each time) with the ice-cold binding buffer. To eliminate any difference between the functional characteristics shown by macrophages on longer incubation periods, all experiments were normally carried out after culturing for 16 h. The binding buffer was prepared by mixing 1 vol. of buffer A [10 mM sodium phosphate, 150 mM NaCl containing 5 mM EDTA (pH 7.4)] and 4 vol. of buffer B [5 mM Tris buffer containing 5% (w/v) glucose and 5 mM EDTA (pH 7.4)], and then 0.05% (w/v) gelatin and 1.5 mg/ml BSA were added. To study the effect of differing ionic strength on the binding, the binding buffer was prepared by mixing buffer A and buffer B in various ratios. The difference between buffers A and B, i.e. 10 mM sodium phosphate versus 5 mM Tris/HCl, does not influence the binding of ^{125}I-SP-D to alveolar macrophages in the presence of buffer containing 30 mM NaCl. After blocking the non-specific attachment sites in the wells with the gelatin–BSA buffer for more than 30 min, the buffer was removed from the plates and the buffer containing ^{125}I-SP-D was added. Unless otherwise stated, the monolayer was incubated for 90 min on ice. After rapid washing with the binding buffer, the amount of bound radiolabel in the wells was determined using a γ-counter.

C1q-binding study

Human C1q was purified from human serum as described by Reid (1981) and was iodinated by the procedure utilizing iodogen (Fraker and Speck, 1978). C1q-binding studies were carried out in a similar manner to the method used for the ^{125}I-SP-D-binding study, except that the binding buffer used was 10 mM PBS (pH 7.4), containing 1 mM EDTA, 5 mg/ml BSA and 0.1% (w/v) gelatin.

RESULTS

Radioiodination of SP-D

Purified SP-D migrated with an apparent molecular mass of

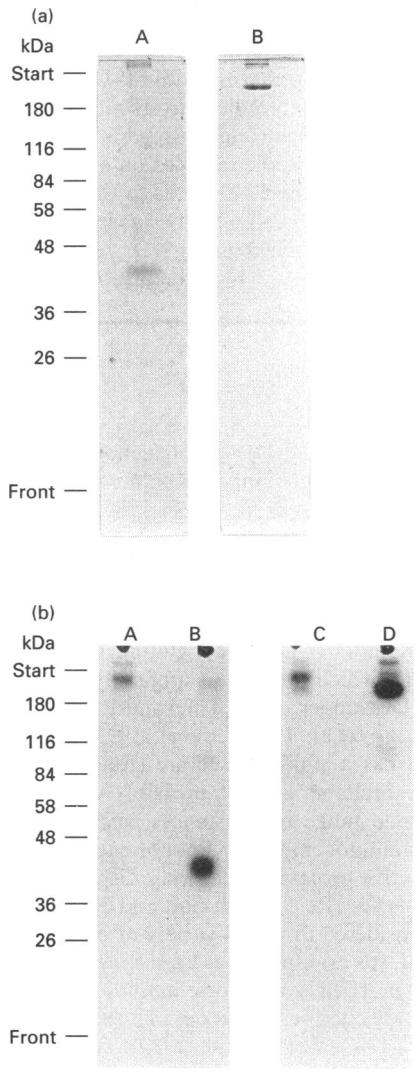

Figure 1 Electrophoretic analysis of SP-D and ^{125}I-SP-D

(**a**) Preparations of purified SP-D were analysed by electrophoresis on 10% (w/v) polyacrylamide gels in the presence of SDS under reducing (lane A) and non-reducing (lane B) conditions and stained with Coomassie Blue. (**b**) ^{125}I-SP-D (30000 c.p.m.) eluted from a maltose–Sepharose column (lanes B and D) and the unbound fraction (30000 c.p.m.) (lanes A and C) were analysed by electrophoresis on 10% (w/v) polyacrylamide gels in the presence of SDS under reducing (lanes A and B) and non-reducing conditions (lanes C and D) and autoradiographed for 12 h. The marks seen at the top of the tracks in (**b**) were made above the application points of the samples in order to clearly identify the lanes during the experiment.

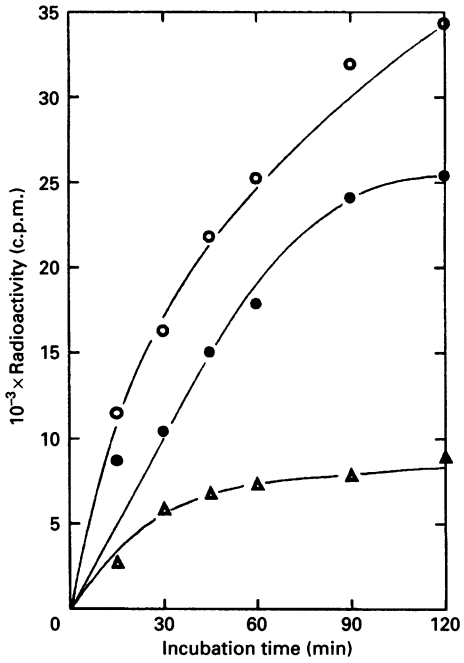

Figure 2 Time-dependent binding of ^{125}I-SP-D by primary cultures of bovine alveolar macrophages

Monolayers of alveolar macrophages were incubated for the indicated time, at 4 °C, in 500 μl of the binding buffer containing 9.5 ng of ^{125}I-SP-D. The cell monolayers were then washed and harvested, and the total binding in the presence of the cells (○), without cells (△) and the calculated cell-specific binding (●) are shown. The results presented are from a representative one of three similar experiments.

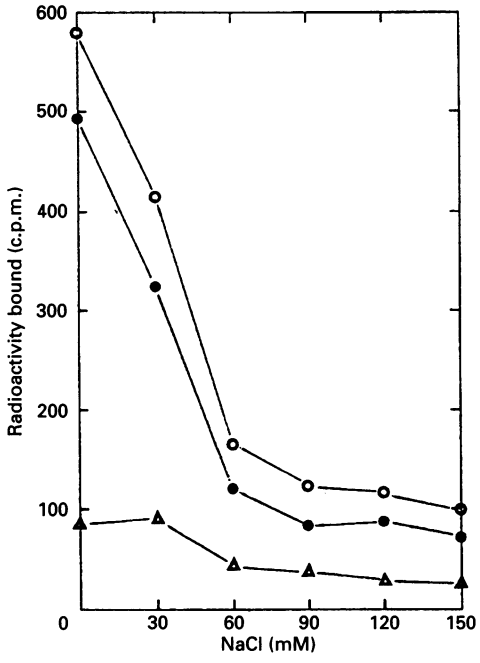

Figure 4 Ionic strength dependency of ^{125}I-SP-D binding to alveolar macrophages

Monolayers of alveolar macrophages were incubated with ^{125}I-SP-D (15 ng/ml) for 90 min at 4 °C. The buffers used were prepared as described in the Materials and methods section. Total binding (○), binding in the absence of the cells (△) and the calculated specific binding (●) were determined. The data presented are from a representative one of three similar experiments.

43 kDa when analysed by electrophoresis under reducing and denaturing conditions. Under non-reducing conditions, the major form of SP-D appeared as a band of apparent molecular mass of 190 kDa in 10% (w/v) SDS/PAGE (Figure 1a) when globular proteins are used as markers. Because of its collagen-like structure, SP-D migrated, under non-reducing conditions, with a higher apparent molecular mass than the 129 kDa predicted for a disulphide-linked trimer of those 43 kDa chains. However, SP-D showed the expected apparent molecular mass of 130 kDa in

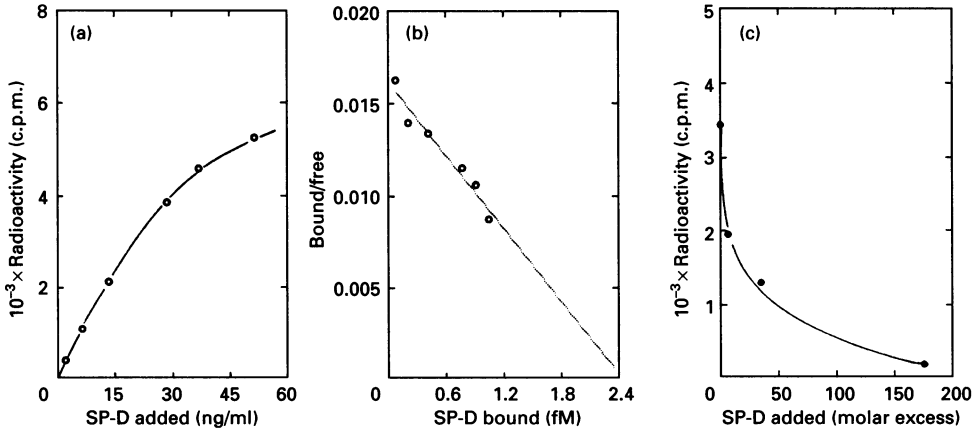

Figure 3 ^{125}I-SP-D binding to bovine alveolar macrophages

(**a**) Concentration-dependent binding of ^{125}I-SP-D to bovine alveolar macrophages. Monolayers of alveolar macrophages were incubated for 2 h, at 4 °C, with increasing concentrations (2–50 ng/ml) of ^{125}I-SP-D in the binding buffer. The cell monolayers were then washed and harvested and the bound radioactivity was quantified in a γ-counter. Results were corrected for non-specific binding by subtracting radioactivity bound in the absence of the cells. Data presented are from a representative one of three similar experiments. (**b**) Scatchard analysis of ^{125}I-SP-D binding to alveolar macrophages. (**c**) Inhibition of ^{125}I-SP-D binding to alveolar macrophages by unlabelled SP-D. Monolayers of alveolar macrophages were incubated for 2 h, at 4 °C, in the presence of ^{125}I-SP-D (20 ng/ml) with increasing amounts of unlabelled SP-D. Data are from a representative one of three similar experiments.

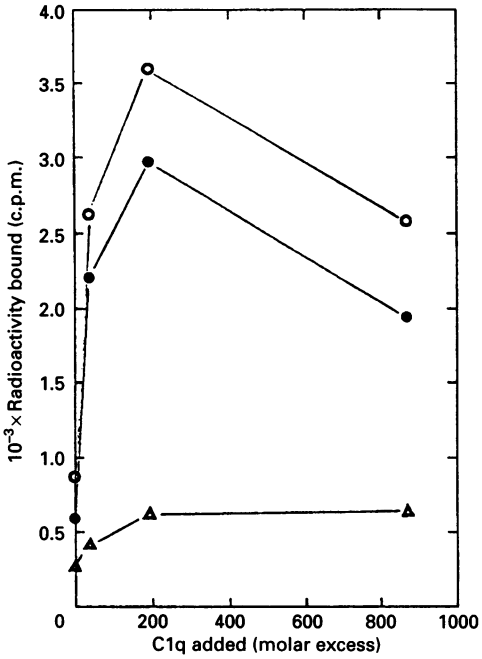

Figure 5 Effect of C1q on ^{125}I-SP-D binding to alveolar macrophages

Monolayers of alveolar macrophages were incubated for 2 h at 4 °C in the presence of ^{125}I-SP-D (17 ng/ml) with increasing amounts of C1q in the binding buffer containing 30 mM NaCl. The total binding in the presence of the cells (○), without cells (△) and the calculated cell-specific binding (●) are shown. The data presented are from a representative one of three similar experiments.

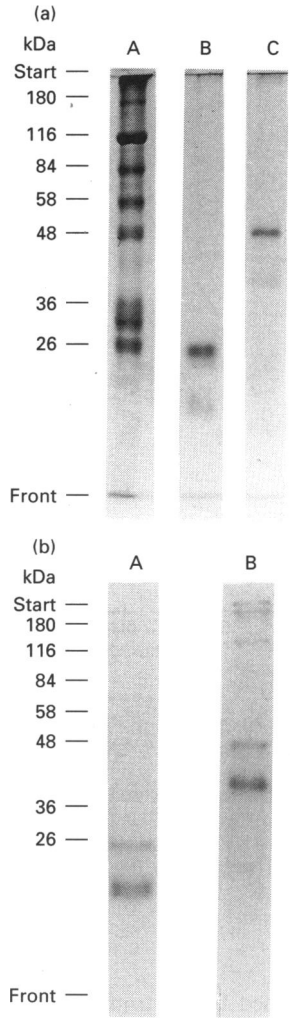

Figure 6 Electrophoretic analysis of C1q and ^{125}I-C1q

(**a**) Preparations of purified C1q were analysed by electrophoresis on 10% (w/v) polyacrylamide gels in the presence of SDS under non-reducing (lane C) and reducing (lane B) conditions. The molecular mass markers are in lane A. (**b**) ^{125}I-C1q were analysed on 10% (w/v) polyacrylamide gels in the presence of SDS under reducing (lane A) and non-reducing (lane B) conditions and autoradiographed for 12 h.

7.5% (w/v) SDS/PAGE under non-reducing conditions, when polypeptides which have a similar overall structure to that of SP-D were used as markers (e.g. the subunits of C1q). ^{125}I-SP-D showed exactly the same behaviour as the unlabelled protein in SDS/PAGE on 10% (w/v) polyacrylamide gels, in both reducing and non-reducing conditions (Figure 1b). The behaviour of the SP-D preparation (before and after labelling with ^{125}I) was assessed by gel-filtration chromatography under non-dissociating conditions on Superose-6, as well as by SDS/PAGE. The results obtained were consistent with the SP-D preparation used in the binding studies, being composed of molecules of 516 kDa containing 12 chains of 43 kDa. The concentration of ^{125}I-SP-D was estimated by a sandwich e.l.i.s.a., as described by Miyamura et al. (1994) and the final specific radioactivity of the protein was 0.1×10^8–1.1×10^8 c.p.m./μg. More than 90% of the radioactivity was precipitated by treatment with 10% (w/v) trichloroacetic acid.

Binding of ^{125}I-SP-D to alveolar macrophages

When monolayers of alveolar macrophages were incubated with ^{125}I-SP-D at 4 °C, a time-dependent binding of radioactivity to the cells was observed (Figure 2). Incubation of various concentrations of ^{125}I-SP-D with the cells revealed a saturable pattern (Figure 3a). The cell-specific binding was calculated by subtracting radioactivity bound in the absence of the cells from the total radioactivity bound in the presence of the cells. Scatchard analysis (Scatchard, 1949) gave a straight line, consistent with a single class of high-affinity binding (Figure 3b). The apparent dissociation constant K_d was $(3.6 \pm 1.3) \times 10^{-11}$ M, assuming that native SP-D is assembled from 12 chains of 43 kDa to give a molecule of 516 kDa (Lu et al., 1993). The ability of excess of unlabelled SP-D to compete with ^{125}I-SP-D binding was examined (Figure 3c). A 200-fold excess of unlabelled SP-D inhibited the cell-specific binding by up to 90%. The binding of ^{125}I-SP-D to the cells was examined in the binding buffer with various salt concentrations (Figure 4). Lysis of macrophages due to hypotonic shock was prevented by the use of a buffer containing 5% (w/v) glucose (see the Materials and methods section). It is known that 5% (w/v) glucose can be used to replace saline [0.9% (w/v) NaCl] in the handling of erythrocytes. Macrophages seem likely to be less susceptible than erythrocytes to hypotonic shock in medium containing glucose. The binding of ^{125}I-SP-D to alveolar macrophages was stronger at lower ionic strengths. The effect of the serum protein C1q on binding was also studied. Enhancement, rather than inhibition, of the binding of ^{125}I-SP-D to macrophages was observed on addition of C1q, in 50–900-fold molar excess over the amount of labelled SP-D used (Figure 5).

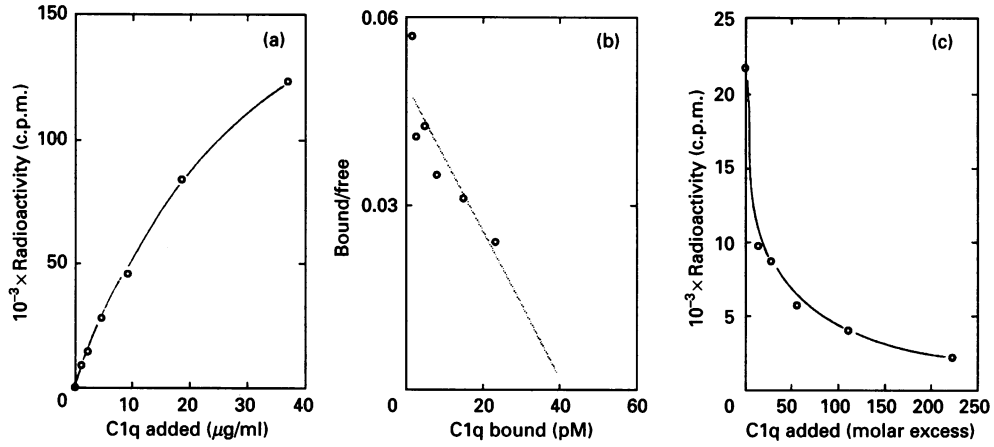

Figure 7 ^{125}I-C1q binding to human alveolar macrophages

(**a**) Concentration-dependent binding. Monolayers of alveolar macrophages were incubated for 90 min, at 4 °C, in 300 μl of the binding buffer containing increasing amount of ^{125}I-C1q (2.3–37 μg/ml). The cell monolayers were then washed and the total binding of ^{125}I-C1q was determined. (**b**) Scatchard analysis. (**c**) Inhibition of ^{125}I-C1q binding to alveolar macrophages by unlabelled C1q. Monolayers of human alveolar macrophages were incubated for 90 min, at 4 °C, in the presence of ^{125}I-C1q (3.7 μg/ml) with increasing amounts of unlabelled C1q. The total binding of ^{125}I-C1q was shown. Specific radioactivity of ^{125}I-C1q used was 0.49×10^6 c.p.m./μg.

Binding of ^{125}I-C1q to alveolar macrophages

The behaviour, on SDS/PAGE, of ^{125}I-labelled C1q used in these experiments is shown in Figure 6b. It should be noted that the ^{125}I label is preferentially incorporated into a tyrosine residue close to the N-terminal end of the C-chain of C1q thus giving preferential labelling of the C–C subunit (in non-reduced samples) or the C-chain (in reduced samples) which are the lower bands (Figure 6b) in the non-reduced (track B) and reduced (track A) samples respectively. With respect to binding to macrophages, both the labelled A–B and C–C subunits are bound to the macrophages as these are linked, by strong non-covalent bonds, to give the intact C1q molecule under the non-dissociating conditions used in the binding experiments. ^{125}I-C1q bound to human alveolar macrophages in a time- (results not shown) and concentration-dependent manner (Figure 7a). Scatchard analysis gave a straight line (Figure 7b) and the apparent dissociation constant K_d was 3×10^{-6} M, assuming that native C1q has a molecular mass of 460 kDa. Addition of a 200-fold excess of unlabelled C1q inhibited the binding by over 90% (Figure 7c).

DISCUSSION

We have provided the evidence that a specific, high-affinity and saturable receptor for SP-D is expressed by the primary cultures of alveolar macrophages. The binding of SP-D to the macrophages did not require Ca^{2+} and is enhanced at lower ionic strengths. As the binding was observed at 4 °C, it would be expected to be due to interaction of SP-D with cell-surface receptors. The apparent dissociation constant is $(3.6 \pm 1.3) \times 10^{-11}$ M for the binding of ^{125}I-SP-D to bovine alveolar macrophages. We obtained a value of 1×10^{-10} M using human alveolar macrophages. These values are closer to the values reported for the lectin binding of SP-D to bacterial polysaccharides (2×10^{-11} M) (Kuan et al., 1992) than to those for SP-A binding to alveolar type-II cells ($5–6.4 \times 10^{-10}$ M) (Kuroki et al., 1988; Wright et al., 1989) or to SP-A binding of the isolated C1q receptor (7.4×10^{-10} M) (Malhotra et al., 1992). Although the binding buffer contained EDTA, at a concentration of 5 mM, it is possible that traces of Ca^{2+} released from the cells could facilitate the lectin binding between SP-D and carbohydrate moieties on the surface of the cells. We, however, found that using 10% (w/v) xylose (a five-carbon ring sugar which would not be expected to show any inhibition of SP-D binding) instead of 5% (w/v) glucose in buffer B did not alter the binding (results not shown), suggesting that the SP-D binding to the cells is not due to lectin activity. Initially, we used a buffer without gelatin for the binding studies. However, to eliminate non-specific binding gelatin had to be added to the binding buffer. The GVB buffer [veronal barbitone-buffered saline containing 0.05% (w/v) gelatin] used for C1q haemolytic studies was used in these studies (Jiang et al., 1991).

The SP-D used in this study was not delipidated, as a substantial amount of the protein was lost after the treatment with the delipidation agent, 1-butanol. SP-D has been reported to bind specifically to phosphatidylinositol, a surfactant lipid (Ogasawara et al., 1992). As the lipid binding required Ca^{2+} and was inhibited by competing saccharides, it was suggested that this interaction involves lectin binding between the inositol moiety and the globular domain of SP-D. Although 5 mM EDTA in the binding buffer should block this specific interaction, non-specific interaction with other lipids has to be considered, as alveolar macrophages take up lipids. Kuroki et al. (1991b) have reported that lipids (mainly phospholipids) are co-purified with SP-D when it is isolated from the 33000 *g* lung lavage supernatant using lectin-affinity chromatography followed by an adsorption step to barium sulphate. Although our purification procedure involves gel-filtration chromatography in the presence of 10 mM EDTA, it does not involve any lipid-extraction step, It is difficult to discern whether the binding shown here has been influenced or even mediated by lipid(s) bound to SP-D. As SP-D loses its binding capability for SP-A after delipidation, it has been suggested that the functionally active form of SP-D may be lipid-bound (Kuroki et al., 1991a). We found that SP-D extracted with 1% (v/v) Triton X-100 showed similar binding properties (K_d 9×10^{-11} M) to that of unextracted SP-D. However, we have no evidence to show that all lipids were removed by this extraction.

SP-A, SP-D, CL-43, conglutinin and MBP comprise a subfamily, group III, of the C-type lectins. All of these proteins have a similar overall structure, i.e. an N-terminal collagen-like domain connected to a globular domain having C-type lectin

activity. This type of structure is similar to that of C1q, the recognition component of the complement classical pathway; however, C1q does not have lectin activity in its globular domains. SP-D and conglutinin have longer collagen-like domains than those of SP-A or C1q (Voss et al., 1991; Lu et al., 1993), and are seen to have a tetrameric oligomer form in the electron microscope, whereas both SP-A and C1q have hexameric oligomer forms. C1q binds to its specific receptor through its collagen-like 'stalk' domain and triggers various immune reactions (Ghebrehiwet, 1987). Although SP-A, CL-43, conglutinin and MBP have been reported to bind to the isolated C1q receptor (Malhotra et al., 1990, 1993; Holmoskov et al., 1993), the binding of SP-D to the C1q receptor has not been shown. In our study, an excess amount of C1q enhanced the binding of ^{125}I-SP-D to alveolar macrophages to approx. 300% of the control value. The role of C1q in the apparent enhancement of binding of ^{125}I-SP-D to alveolar macrophages, in the presence of unlabelled C1q, is not clear. It seems unlikely to be a result of a direct interaction between the two proteins as no binding of SP-D to C1q was observed using an assay in which ^{125}I-SP-D was used to detect solid-phase C1q, and vice versa, in 10 mM potassium phosphate buffer, pH 7.4, containing 1 mg/ml BSA. Involvement of the C1q receptor was also considered a possible reason for the enhanced binding of ^{125}I-SP-D to the alveolar macrophages. Therefore purified C1q receptor was coated on to microtitre plates and the binding of ^{125}I-SP-D to the C1q receptor was examined, in gelatin-containing buffers, in the presence and absence of C1q. Some binding of ^{125}I-labelled SP-D was seen in the presence of C1q but it did not appear likely to fully account for the increased levels of ^{125}I-SP-D binding seen in the experiments involving the addition of unlabelled C1q to alveolar macrophages. On the other hand, we have shown the specific binding of ^{125}I-labelled human C1q to bovine alveolar macrophages could be inhibited by unlabelled human C1q (Figure 7). The binding of radiolabelled bovine C1q to bovine alveolar macrophages was inhibited by 90% by addition of a 200-fold excess of unlabelled bovine C1q using buffer containing 30 mM NaCl (L. E. A. Leigh and K. Miyamura, unpublished work). The apparent dissociation constant is 3×10^{-6} M for the binding of ^{125}I-human C1q to bovine alveolar macrophages. This value is in good agreement with that previously reported by Sorvillo et al. (2×10^{-6} M) for the binding of human C1q to the monolayers of human blood monocytes (Sorvillo et al., 1986), and is quite different from the value we have shown for SP-D binding to alveolar macrophages. Based on these observations, we conclude that receptor involved in the SP-D binding is different from that utilized by C1q. However, more data are required to understand the enhancing effect of C1q on the SP-D binding to macrophages.

The specific binding of SP-A to alveolar macrophages has been shown to be inhibited by C1q and type-V collagen (Pison et al., 1992); to have a stimulatory effect on chemotaxis of the cells (Wright and Youmans, 1993); and to enhance free-radical production in the cells (van Iwaarden et al., 1990). However, there is no direct evidence that binding of SP-A to the C1q receptor mediates each of these biological functions. Pison et al. (1992) have reported a K_d of 4×10^{-9} M for the binding of rat ^{125}I-SP-A to rat alveolar macrophages. In the case of SP-D, it has been reported that it does enhance free-radical production, to the same degree as SP-A does, on binding to alveolar macrophages (van Iwaarden et al., 1992a). However, it is unknown whether or not SP-D binds to the same receptor on alveolar macrophages and mediates its effect by the same mechanism(s) used by SP-A.

Although we have shown specific binding of SP-D to alveolar macrophages, the primary biological function of SP-D is not known yet. SP-D has been reported to bind to surface lipopolysaccharides of Gram-negative bacteria (Kuan et al., 1992) via the carbohydrate-binding properties in its C-type lectin globular domains. The complex of bacteria plus bound SP-D may be presented to macrophages, via the collagen-like regions of SP-D, at specific receptors on the macrophage cell surface.

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