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Elastase Controls the Binding of the Vitamin D-Binding Protein (Gc-Globulin) to Neutrophils: A Potential Role in the Regulation of C5a Co-Chemotactic Activity

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The vitamin D-binding protein (DBP) binds to the plasma membranes of numerous cell types and mediates a diverse array of cellular functions. DBP bound to the surface of leukocytes serves as a co-chemotactic factor for C5a, significantly enhancing the chemotactic activity of pM concentrations of C5a. This study investigated the regulation of DBP binding to neutrophils as a possible key step in the process of chemotaxis enhancement to C5a. Using radioiodinated DBP as a probe, neutrophils released 70% of previously bound DBP into the extracellular media during a 60-min incubation at 37°C. This was suppressed by serine protease inhibitors (PMSF, Pefabloc SC), but not by metallo- or thiol-protease inhibitors. DBP shed from neutrophils had no detectable alteration in its m.w., suggesting that a serine protease probably cleaves the DBP binding site, releasing DBP in an unaltered form. Cells treated with PMSF accumulate DBP vs time with over 90% of the protein localized to the plasma membrane. Purified neutrophil plasma membranes were used to screen a panel of protease inhibitors for their ability to suppress shedding of the DBP binding site. Only inhibitors to neutrophil elastase prevented the loss of membrane DBP-binding capacity. Moreover, treatment of intact neutrophils with elastase inhibitors prevented the generation of C5a co-chemotactic activity from DBP. These results indicate that steady state binding of DBP is essential for co-chemotactic activity, and further suggest that neutrophil elastase may play a critical role in the C5a co-chemotactic mechanism.


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Neutrophil elastase, also known as human leukocyte elastase (E.C.3.4.21.37), has been shown to cleave several CD Ags (CD14, CD16, CD43, CD44), releasing a soluble form into the extracellular media (32, 33). Although the majority of neutrophil elastase is stored in azurophil granules, several reports have demonstrated active enzyme on the cell surface (34–37). An earlier report from our laboratory has shown that, at 37°C, neutrophils shed DBP from the plasma membrane into the extracellular media (26). Moreover, the loss of DBP from the cell surface is correlated temporally with the decay in C5a co-chemotactic activity (26). These results suggested that either DBP or its binding site is proteolytically processed by neutrophils. The goal of the present study was to characterize the regulation of DBP binding to human neutrophils. The results demonstrate that elastase is needed for steady state binding of DBP to neutrophils. Specific elastase inhibitors disrupt steady state binding, which causes DBP to accumulate on the plasma membrane and suppresses co-chemotactic activity for C5a.

Materials and Methods

Reagents

Human rC5a was a generous gift from Karl Mollison of Abbott Laboratories (Abbott Park, IL), and was prepared using an Escherichia coli expression system, as previously described (38). Purified human DBP was purchased from Biodesign International (Kennebunkport, ME). The detergent Triton X-100 was purchased from Sigma (St. Louis, MO). Protease inhibitors were purchased from the following sources: 3,4-dichloroisocoumarin, PMSF, 1,10-phenanthroline, N-methoxysuccinyl-alal-ala-pro-alal-chloromethyl ketone (AAPA-CMK), N-methoxy succinyl-alal-ala-val-chloromethyl ketone (AAPV-CMK), trans-epoxysuccinyl-l-lleucylamido(4-guanidino)butane (E-64), leupeptin, and pepstatin A were purchased from Sigma; tosyl-l-lysine-chloromethyl ketone and tosyl-l-phenylalanine-chloromethyl ketone were from Bachem (Torrance, CA); p-phenylalanine-l-proline-l-arginine-chloromethyl ketone (FPR-CMK) and p-phenylalanine-µ-phenylalnine-l-arginine-chloromethyl ketone (FFR-CMK) were purchased from Calbiochem (San Diego, CA); chymostatin and 4-(2-aminoethyl)-benzenesulfonyl fluoride (Pefabloc SC) were from Roche Molecular (Indianapolis, IN); carboxyoxy-glycine-leucine-phenylalanine-chloromethyl ketone (Z-GLF-CMK) was from Enzyme Systems Products (Livermore, CA); and recombiant human secretory leukocyte protease inhibitor (SLPI) was obtained from R&D Systems (Minneapolis, MN).

Isolation of human neutrophils and neutrophil plasma membranes

Neutrophils were isolated from the venous blood of healthy, medication-free, paid volunteers (who gave informed consent) using a standard three-step isolation procedure described previously (26). Subcellular fractionation of neutrophils and isolation of purified plasma membranes have been described in detail previously (27).

Radiiodination of DBP

Purified DBP (200 µg) was labeled using one Iodobead (Pierce, Rockford, IL) and 1 mCi of Na125I (DuPont-NEN, Wilmington, DE) for 5 min. The reaction was terminated by removing the solution from the Iodobead. Free Na125I was separated from 125I-labeled DBP (125I-DBP) by gel filtration on a PD-10 (Sephadex G-25; Pharmacia-LKB, Piscataway, NJ) column. Purified human DBP was purchased from Abbott (Abbott Park, IL) and was prepared using an Escherichia coli expression system. 3,4-dichloroisocoumarin, PMSF, 1,10-phenanthroline, N-methoxysuccinyl-alal-ala-pro-alal-chloromethyl ketone (AAPA-CMK), N-methoxy succinyl-alal-ala-val-chloromethyl ketone (AAPV-CMK), trans-epoxysuccinyl-l-lleucylamido(4-guanidino)butane (E-64), leupeptin, and pepstatin A were purchased from Sigma; tosyl-l-lysine-chloromethyl ketone and tosyl-l-phenylalanine-chloromethyl ketone were from Bachem (Torrance, CA); p-phenylalanine-l-proline-l-arginine-chloromethyl ketone (FPR-CMK) and p-phenylalanine-µ-phenylalnine-l-arginine-chloromethyl ketone (FFR-CMK) were purchased from Calbiochem (San Diego, CA); chymostatin and 4-(2-aminoethyl)-benzenesulfonyl fluoride (Pefabloc SC) were from Roche Molecular (Indianapolis, IN); carboxyoxy-glycine-leucine-phenylalanine-chloromethyl ketone (Z-GLF-CMK) was from Enzyme Systems Products (Livermore, CA); and recombiant human secretory leukocyte protease inhibitor (SLPI) was obtained from R&D Systems (Minneapolis, MN).

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Quantitative binding assay

The binding of radiiodiinated DBP to intact neutrophils has been described previously (26). Radiolabel binding to purified plasma membranes was measured using a vacuum filtration manifold and 0.1-µm pore-size Durapore type VV filters (Millipore), as described previously (27).

Preparation of neutrophil detergent lysates

Detergent lysates of neutrophils were prepared by adding 100 µl of 1% Triton X-100, 50 mM HEPES (pH 7.4) containing 20 mM benzamidine, 10 mM EDTA, 10 mM NaCl, as well as the following inhibitors added fresh immediately before lysis: 2 mM PMSF, 2 mM 1,10-phenanthroline, 0.5 mM E-64, 0.2 mM 3,4-dichloroisocoumarin, 0.1 mM leupeptin, and 0.1 mM pepstatin. Lysates were vortexed thoroughly until all particulate matter was solubilized (usually 5–10 s) and then placed at 37°C for 60 min. The detergent-insoluble material was then pelleted by centrifuging the lysates in a microfuge for 10 min at 15,000 × g at 4°C.

Chemotaxis assay

Cell movement was quantitated using a 48-well microchemotaxis chamber (Neuroprobe, Cabin John, MD) and 5-µm pore-size cellulose nitrate filters (Toyo, purchased from Neuroprobe), as previously described (26).

PAGE and autoradiography

Samples were separated by PAGE in the presence of SDS (SDS-PAGE) using the discontinuous buffer system described by Laemmli (39). Samples were prepared for electrophoresis by boiling (100°C) for 7 min with an equal volume of electrophoresis sample buffer (0.125 M Tris, pH 6.8, 20% glycerol, 4% SDS) containing 0.2 M DTT as the reducing agent. After electrophoresis, the gels were stained, destained, dried, and exposed to x-ray film at −80°C.

Data analysis and statistics

A minimum of three experiments was performed for each assay. Results of several experiments were analyzed for significant differences among group means using the Newman-Keul’s Multiple Comparisons test using a statistical software program (InSTAT).

Results

In an earlier report, we observed that neutrophils (at 37°C) shed previously bound DBP from the plasma membrane into the extracellular media (26). To determine whether a specific class of cell surface proteases mediated this effect, neutrophils were treated with inhibitors specific to either metallo (1,10-phenanthroline)-, thiol (E-64)-, or serine proteases (PMSF, Pefabloc SC), and the amount of previously bound radiiodinated DBP was measured in the cell-free supernatant. Fig. 1A shows that ~70% of previously bound DBP is released from cells during a 60-min incubation at 37°C. Treatment of cells with thiol- or metalloprotease inhibitors had no effect on the amount of DBP shed. In contrast, the serine protease inhibitor PMSF, or its water soluble analogue Pefabloc SC, significantly suppressed the amount of DBP released by neutrophils (Fig. 1A). Radiolabeled DBP released into the cell-free supernatant also was examined by SDS-PAGE to determine whether the protein was cleaved. Fig. 1B shows that 125I-DBP shed from neutrophils has no detectable alteration in its m.w. These results demonstrate that a serine protease probably cleaves the binding site, releasing DBP in an unaltered form.

Because the serine protease inhibitor PMSF was very effective at preventing neutrophils from shedding DBP into the extracellular media, it follows that cells pretreated with PMSF should accumulate radiolabeled DBP over time. Fig. 2 demonstrates that neutrophils pretreated with 0.5 mM PMSF and then incubated with 125I-DBP accumulate the protein in a linear manner vs time. In contrast, the level of cell-associated 125I-DBP in sham-treated (control) neutrophil plateaus between 30 and 60 min at 35–40 fmol DBP/106 cells, very similar to what has been reported previously (26). Furthermore, subcellular fractionation revealed that almost 90% of the cell-associated DBP remained with the plasma membrane fraction in both PMSF-treated and control cells (data not shown) (27). At the concentrations of PMSF used (0.5 mM), greater than 95% of cells were viable, as determined by trypan blue dye exclusion and the release of lactate dehydrogenase (data not shown), consistent with our previous findings (40).

The neutrophil DBP binding site, a chondroitin sulfate proteoglycan, localizes to the detergent-insoluble fraction of cells solubilized using Triton X-100 (27). Therefore, when DBP is bound to
HBSS were incubated with 100 nM 125I-DBP for 45 min at 37°C in buffer.

Data are expressed as the percentage of total counts in the cell-free supernatant. After the first incubation, cells contained 38 fmol DBP/10^6 neutrophils. Following the second incubation with protease inhibitors, the cells contained the following amounts of DBP/10^6 neutrophils: control (12.5 fmol), E-64 (13 fmol), 1,10-phenanthroline (11.5 fmol), Pefabloc (27.5 fmol), and PMSF (30 fmol). The results represent the mean ± SEM of three experiments performed in duplicate using different donors. Values for Pefabloc- and PMSF-treated cells were significantly less (p < 0.001) than all other groups.

FIGURE 1. Inhibition of serine proteases causes a decrease in the percentage of DBP that is shed from neutrophils. Purified neutrophils (10^6) in HBSS were incubated with 100 nM 125I-DBP for 45 min at 37°C in buffer (HBSS). After 45 min, cells were pelleted, washed, and resuspended in HBSS containing E-64 (0.5 mM), 1,10-phenanthroline (1 mM), Pefabloc (0.5 mM), or PMSF (0.5 mM), and were incubated another 60 min at 37°C. A. After this second incubation, the cells were pelleted and both the cellular pellet and the cell-free supernatant were counted for radioactivity. Data are expressed as the percentage of total counts in the cell-free supernatant. After the first incubation, cells contained 38 fmol DBP/10^6 neutrophils. Following the second incubation with protease inhibitors, the cells contained the following amounts of DBP/10^6 neutrophils: control (12.5 fmol), E-64 (13 fmol), 1,10-phenanthroline (11.5 fmol), Pefabloc (27.5 fmol), and PMSF (30 fmol). The results represent the mean ± SEM of three experiments performed in duplicate using different donors. Values for Pefabloc- and PMSF-treated cells were significantly less (p < 0.001) than all other groups. B. After the second incubation, the cell-free supernatant was collected and analyzed by SDS-PAGE under reducing conditions. Approximately equal counts were added to each lane, and a representative autoradiogram is shown. Lane 1, 125I-DBP that was not incubated with cells (control); lane 2, cell-free supernatant from untreated cells; lane 3, cell-free supernatant from PMSF-treated neutrophils.

its proteoglycan binding site, the complex partitions with the Triton X-100-insoluble fraction. Fig. 3 shows that neutrophils accumulate DBP in the detergent-insoluble fraction with increasing concentrations of PMSF, indicating that inhibition of a serine protease results in the accumulation of DBP binding site complexes. Previously, we reported that neutrophils bind 5-fold less radiolabeled albumin than DBP (26). Treatment of neutrophils with 0.5 mM PMSF does not permit neutrophils to accumulate albumin, and there is no shifting of the protein to the detergent-insoluble pellet, indicating that the effect on DBP binding is not a generalized consequence of PMSF treatment (data not shown). Figs. 2 and 3 indicate that inhibition of a neutrophil serine protease disrupts the steady state balance of DBP binding/shedding, which results in its accumulation on the cell surface.

The role of serine proteases in the regulation of cellular DBP binding was characterized further using purified neutrophil plasma membranes. Plasma membranes were used instead of intact cells to avoid the potential problem of up-regulation of enzymes from intracellular granules. Previously, we have shown that the binding of 125I-DBP to neutrophil plasma membranes essentially is identical with intact cells, demonstrating that membrane preparations are a good model system to investigate DBP binding (27). Plasma membranes should have a fixed number of DBP binding sites and serine protease activity, and thus, one would predict that there would be no steady state binding. Indeed, Fig. 4A shows that the binding of 125I-DBP to neutrophil plasma membrane (2°C) plateaus at 60 min between 17 and 18 fmol/μg membrane protein. In contrast, the maximal amount of DBP bound to membranes at 37°C peaks at 20 min and only was 65% of the maximal amount bound at 2°C. Continued incubation at 37°C resulted in a rapid diminution of 125I-DBP binding, with a complete loss in the binding capacity of membranes at 120 min (Fig. 4A). Addition of PMSF to the membranes prevents the loss in DBP-binding capacity at 37°C by more than 90% (data not shown). The foregoing data raise the question, does DBP need to be bound in order for a serine protease to inactivate its binding site? To address this question, plasma membranes were preincubated for various times at 37°C, after which radiolabeled DBP was added and the binding assay was performed on ice (2°C). Fig. 4B clearly shows that degradation of the DBP-binding capacity of plasma membranes is constitutive and does not require DBP bound to its binding site. These results demonstrate that the constitutive activity of a cell surface serine protease reduces the binding capacity of plasma membranes for DBP.

Neutrophils possess several serine esterases, including elastase, cathepsin G, proteinase 3, and urokinase-type plasminogen activator (41). In addition, plasma-derived serine proteases such as kallikrein, plasmin, and thrombin can bind to neutrophils, and their proteolytic activity can be detected on the cell surface (42–44). Thus, there are several serine esterases that potentially could cleave the plasma membrane DBP binding site. To identify the responsible serine protease, plasma membranes were treated with several selective inhibitors and then were assessed for their capacity to bind radiolabeled DBP. Fig. 5 clearly shows that inhibitors of neutrophil elastase (AAPA-CMK and AAPV-CMK), neutrophil serine proteases (SLPI), as well as the serine class-specific inhibitors PMSF and Pefabloc SC significantly increase the binding of DBP to plasma membranes over the untreated controls. In addition, similar treatment of intact neutrophils produced almost identical results (data not shown). These results implicate membrane-bound neutrophil elastase as the protease that cleaves the DBP binding site.

FIGURE 2. Neutrophils treated with PMSF accumulate DBP vs time. Neutrophils (10^6 cells) were pretreated with either buffer (HBSS) or 0.5 mM PMSF for 10 min at 22°C. Cells then were incubated in HBSS with 100 nM 125I-DBP for the designated time at 37°C. The cells were washed three times in ice-cold HBSS, then counted for total cell-associated radioactivity. Data are expressed as fmol of DBP associated per million neutrophils. The data represent the mean ± SEM of four to seven separate experiments performed in duplicate using cells from different donors. Values for 30-, 60-, 120-, and 180-min PMSF-treated cells were significantly greater than control cells (p < 0.01 for 30-min sample; p < 0.001 for 60-, 120-, and 180-min samples).
FIGURE 3. PMSF causes DBP to accumulate in the Triton X-100-insoluble fraction. Purified neutrophils were incubated in HBSS with 100 nM 125I-DBP and the designated concentration of PMSF for 45 min at 37°C. The cells were washed in ice-cold HBSS, then lysed in 1% Triton X-100 (containing a complete protease inhibitor cocktail) for 1 h at 37°C. The soluble and insoluble fractions were separated by centrifugation. Data are expressed as fmol of DBP associated per million neutrophils. The data represent the mean ± SEM of three to five separate experiments performed in duplicate using cells from different donors. Percentage of counts in the Triton X-100-insoluble pellet was significantly greater than control (p < 0.001) for 0.2, 0.3, and 0.5 mM PMSF-treated cells.

Finally, the effect of elastase inhibitors on the ability of neutrophils to generate C5a co-chemotactic activity from DBP was examined. Previously, we reported that cell surface binding of DBP is temporally correlated with the generation of C5a co-chemotactic activity (26). Therefore, if the cell surface binding of DBP is perturbed, it follows that the co-chemotactic activity should be altered. Fig. 6 demonstrates that pretreatment of neutrophils with either synthetic chloromethyl ketone-based inhibitors (AAPA-CMK, AAPV-CMK), or an endogenous protein inhibitor (SLPI) prevents the generation of C5a co-chemotactic activity from DBP. In contrast, inhibitors of neutrophil cathepsin G (Z-GLF-CMK and α1-antichymotrypsin) had no effect on co-chemotactic activity. None of the inhibitors altered neutrophil chemotaxis to an optimal concentration of C5a (1 nM); both control and inhibitor-treated cells migrated an average of 65 ± 4 μm/30 min. These results indicate that the steady state binding of DBP is essential for co-chemotactic activity, and further suggest that neutrophil elastase may play a critical role in the C5a co-chemotactic mechanism.

Discussion
The current level of understanding of extracellular chemotactoactic regulatory factors has lagged far behind that of the chemotactic factors they regulate. Moreover, the physiological significance of these factors is not widely appreciated. Indeed, the initial descriptions of a C5a co-chemotactic factor in serum were reported more than 20 years ago (45–47). Identification of DBP as the serum-derived C5a co-chemotactic factor was described in 1988 (11, 12), and subsequently confirmed by several other groups (13–16). However, the mechanism by which DBP enhances chemotaxis to C5a is still unknown. DBP does not alter neutrophil C5a receptor number or Kd for C5a (16, 48) (R.R.K., unpublished observations), thereby discounting the most obvious explanation for its co-chemotactic effect. We believe that the key to uncovering the co-chemotactic mechanism first lies in understanding how DBP interacts with its plasma membrane binding site. It is clear that DBP needs to be bound to its cell surface binding site (a chondroitin sulfate proteoglycan) to mediate the co-chemotactic effect for C5a (26, 49). Furthermore, neutrophils (as well as monocytes, U937 cells, and HL-60 cells) not only bind DBP, but spontaneously shed the protein into the extracellular media (26) (R.R.K., unpublished observations). Therefore, the aim of the present study was to investigate the regulation of DBP binding to human neutrophils.

The results reported in this study show that neutrophil elastase controls the amount of DBP bound to cells by shedding its binding site. A previous report has shown that the binding of 125I-DBP (at 2°C) to intact neutrophils or plasma membranes is nonsaturable vs increasing concentration of the ligand up to 5 μM (27). However, at 37°C, the cellular levels of DBP, at any single concentration, will plateau with time (Fig. 2) (26), probably reflecting a steady state between binding and shedding of DBP on the plasma membrane. Moreover, inhibition of a serine protease (i.e., elastase), by inhibitors or low temperature, disrupts the balance, allowing DBP to accumulate on the cell surface bound to a chondroitin sulfate proteoglycan (Figs. 2 and 3) (27). However, at 37°C, the cellular levels of DBP, at any single concentration, will plateau with time (Fig. 2) (26), probably reflecting a steady state between binding and shedding of DBP on the plasma membrane. Therefore, the aim of the present study was to investigate the regulation of DBP binding to human neutrophils.
FIGURE 5. Binding of DBP to plasma membranes in the presence of various protease inhibitors. A total of 10 µg of plasma membranes was incubated in HBSS-0.1% BSA containing 100 nM 125I-DBP with either no protease inhibitors or one of the following: PMSF (0.5 mM), Pefabloc SC (0.5 mM), AAPV-CMK (50 µM), AAPA-CMK (50 µM), Z-GLF-CMK (50 µM), tosyl-L-phenylalnine-chloromethyl ketone (50 µM), tosyl-L-lysine-chloromethyl ketone (50 µM), FPR-CMK (50 µM), FFR-CMK (50 µM), chymostatin (50 µM), 1,10-phenanthroline (200 µM), E-64 (50 µM), and SLPI (3 µM). Incubations were for 20 min at 37°C. The samples then were separated by vacuum filtration and washed, and the filters were counted for radioactivity. Data are expressed as a percentage increase of DBP bound over the control membranes (11 ± 2.1 fmol DBP bound per µg membrane protein), which were not treated with inhibitors. Data represent the mean ± SEM of three to five separate experiments using membrane preparations from different neutrophil donors. Values for SLPI, PMSF, Pefabloc, AAPA-CMK, and AAPV-CMK are significantly greater (p < 0.01) than all other samples.

Several potential elastase and cathepsin G cleavage sites (50). Third, Fig. 4B verifies that elastase constitutively degrades the binding site. Finally, we have reported earlier that purified elastase treatment of neutrophil plasma membranes could reduce the DBP-binding capacity by >90%; however, purified cathepsin G had no effect on DBP binding (27).

Several reports have demonstrated that the shedding of cell surface molecules is often mediated by more than one protease, including a combination of serine and metalloenzymes (32, 31). Fig. 1A shows that PMSF or Pefabloc treatment does not completely prevent DBP shedding, perhaps suggesting other enzymes act on the binding site. However, we feel that this incomplete inhibition of shedding is due to the nature of the protease inhibitors (Pefabloc and PMSF) and the use on intact neutrophils. Pefabloc SC is a water-soluble molecule that inhibits proteases in aqueous solution much more effectively than membrane-bound enzymes. Conversely, PMSF is unstable in aqueous solutions at pH 7.4 and 37°C (51). Perturbation of neutrophils, such as the purification protocol followed by a 60-min incubation at 37°C, can induce a fusion of intracellular granules with the plasma membrane, which releases proteases from their internal stores (52, 53). Thus, during the 60-min incubation at 37°C, there was probably an up-regulation of surface-bound elastase from intracellular stores that was not inhibited and subsequently degraded the DBP binding site.

The panel of protease inhibitors employed in Fig. 5 clearly implicates elastase as the serine protease mediating the shedding of the DBP binding site. Selective elastase inhibitors AAPA-CMK and AAPV-CMK as well as SLPI were effective in preventing the loss of DBP-binding capacity of neutrophil plasma membranes. Moreover, the inhibitors that were not effective at deterring DBP shedding also do not inhibit elastase. Inhibitors specific for cathepsin G (Z-GLF-CMK), kallikrein (FPR-CMK), and thrombin (FPR-CMK) could not prevent shedding. Aprotinin, an effective plasmin and kallikrein inhibitor, also was used, but had no effect (data not shown). Proteinase 3 (also known as myeloblastin) has a similar substrate specificity as elastase and is inhibited by many of the same reagents. To discriminate between these two proteases, SLPI and AAPA-CMK were employed. Both are effective inhibitors of elastase, but do not inhibit proteinase 3 (54, 55). Therefore, if proteinase 3 were responsible for degrading the DBP binding site, SLPI and AAPA-CMK should have shown no increase in binding over the untreated control. SLPI was the most effective inhibitor at preventing degradation of the DBP-binding capacity of plasma membranes. Perhaps this is because the low molecular mass SLPI (11.7 kDa) is able to inhibit membrane-bound elastase (35, 56).

Clearly, DBP binding to cells is not mediated by a specific high affinity receptor, but rather by nonspecific low affinity binding sites, such as proteoglycans (27) and clearance/scavenger receptors (28). Furthermore, multiple DBP molecules probably bind per mole of binding site (proteoglycan), and we have shown that DBP bound to neutrophil plasma membranes can oligomerize (27). However, there is some selectivity in the interaction of DBP with neutrophils because its binding characteristics are distinctly different from that of human albumin (data not shown) (26), even though DBP is part of the albumin gene family and both proteins share considerable amino acid and structural similarity (50). Neutrophils bind 5-fold less albumin than DBP (26). Moreover, PMSF treatment has no effect on the total amount of albumin bound to cells and does not cause a redistribution of the protein into the detergent-insoluble fraction (Figs. 2 and 3, and data not shown). The precise identity of the chondroitin sulfate proteoglycan that binds DBP is not known, although it is possible that DBP could bind to several different chondroitin sulfate proteoglycans or perhaps any glycosaminoglycan-containing macromolecule. Currently, this is an area of active investigation in our laboratory.

It is not clear how DBP may induce a co-chemotactic response to C5a, but it may involve proteoglycan-mediated clustering signaling components on the cytosolic side of the plasma membranes (57). DBP binding and shedding on the neutrophil plasma membrane correlate temporally with generation (binding) and decay (shedding) of C5a co-chemotactic activity (26). One might speculate that DBP binds to elastase-rich, proteoglycan microdomains on the extracellular face of the plasma membrane and triggers an assembly of intracellular signaling components that facilitates C5a-induced chemotaxis. Elastase may function to terminate the
signal by shedding the DBP binding site complex, and permit a constant dynamic interaction between DBP and its cell surface binding site. Interestingly, cell surface proteoglycans have been shown to play a role in enhancing chemotactic responses to basic fibroblast growth factor (58) and chemokines (59) by binding and localizing the molecules on the cell surface. In addition, the cationic neutrophil elastase is known to electrostatically interact with anionic sulfated proteoglycans (60), and this may serve to localize the enzyme on the plasma membrane. Indeed, it has been demonstrated recently that elastase localizes to the migrating front (pseudodop) of neutrophils responding to a gradient of platelet-activating factor (37). Chemokine receptor complexes, including the C5a receptor, have been shown to cluster in the migrating front of leukocytes (61–63). However, we have never observed DBP-C5a receptor complexes by either co-immunoprecipitation or chemical cross-linking (R.R.K., unpublished observations), suggesting that the two proteins do not interact. Nevertheless, the possibility that DBP induces membrane clustering, without interacting with the C5a receptor, remains to be tested.

DBP is a ubiquitous protein in vivo; it has been detected in almost all body fluids at levels capable of inducing co-chemotaxis to C5a (20). Moreover, no homozygous deficiency of DBP has been reported in any mammal, although a DBP−/− strain of mice recently has been generated (64). Thus, DBP probably would be present any time C5a is produced and would be available to mediate its co-chemotactic effect. A better understanding of the regulation of DBP binding to its cell surface site is necessary to understand the co-chemotactic effect, as well as the other diverse cellular functions of DBP.

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