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Platelet-Derived Thrombospondin-1 Is Necessary for the Vitamin D-Binding Protein (Gc-Globulin) to Function as a Chemotactic Cofactor for C5a1

Glenda Trujillo and Richard R. Kew2

The chemotactic activity of C5a and C5a des Arg can be enhanced significantly by the vitamin D-binding protein (DBP), also known as Gc-globulin. DBP is a multifunctional 56-kDa plasma protein that binds and transports several diverse ligands. The objective of this study was to investigate the mechanisms by which DBP functions as a chemotactic cofactor for C5a using neutrophils and U937 cells transfected with the C5aR (U937-C5aR cells). The results demonstrate that U937-C5aR cells show C5a chemotactic enhancement only to DBP in serum, but, unlike mature neutrophils, this cell line cannot respond to DBP in plasma or to purified DBP. Analysis by SDS-PAGE and isoelectric focusing revealed no structural difference between DBP in serum compared with DBP in plasma. However, plasma supplemented with either serum, DBP-depleted serum, or activated platelet releasate provides a required factor and permits DBP to function as a chemotactic cofactor for C5a. Fractionation of activated platelet releasate revealed that the additional factor possessed the properties of thrombospondin-1 (TSP-1). Finally, purified TSP-1 alone could reproduce the effect of serum or platelet releasate, whereas Abs to TSP-1 could block these effects. These results provide clear evidence that TSP-1 is needed for DBP to function as a chemotactic cofactor for C5a. The Journal of Immunology, 2004, 173: 4130–4136.

1 This investigation was supported in part by National Institutes of Health Grant GM 63769 (to R.R.K.). G.T. was supported by a W. Burghardt Turner Fellowship and the National Science Foundation-funded AGEP Program (both at Stony Brook University). Glenda Trujillo and Richard R. Kew 2 Address correspondence and reprint requests to Dr. Richard R. Kew, Department of Pathology, Stony Brook University School of Medicine, Stony Brook, NY 11794-8691. E-mail address: rkew@notes.cc.sunysb.edu

2 Department of Pathology, Stony Brook University School of Medicine, Stony Brook, NY 11794-8691. Received for publication May 12, 2004. Accepted for publication June 18, 2004. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

3 Abbreviations used in this paper: DBP, vitamin D-binding protein; TSP-1, thrombospondin-1; CVF, cobra venom factor; IEF, isoelectric focusing; ZAS, zymosan-activated serum; ZAP, zymosan-activated plasma; pl, isoelectric point.

The chemotactic activity of the C5-derived peptides can be enhanced significantly by the vitamin D-binding protein (DBP), also known as Gc-globulin. DBP is a multifunctional 56-kDa plasma protein that binds and transports several diverse ligands. The objective of this study was to investigate the mechanisms by which DBP functions as a chemotactic cofactor for C5a using neutrophils and U937 cells transfected with the C5aR (U937-C5aR cells). The results demonstrate that U937-C5aR cells show C5a chemotactic enhancement only to DBP in serum, but, unlike mature neutrophils, this cell line cannot respond to DBP in plasma or to purified DBP. Analysis by SDS-PAGE and isoelectric focusing revealed no structural difference between DBP in serum compared with DBP in plasma. However, plasma supplemented with either serum, DBP-depleted serum, or activated platelet releasate provides a required factor and permits DBP to function as a chemotactic cofactor for C5a. Fractionation of activated platelet releasate revealed that the additional factor possessed the properties of thrombospondin-1 (TSP-1). Finally, purified TSP-1 alone could reproduce the effect of serum or platelet releasate, whereas Abs to TSP-1 could block these effects. These results provide clear evidence that TSP-1 is needed for DBP to function as a chemotactic cofactor for C5a. The Journal of Immunology, 2004, 173: 4130–4136.

C omplement activation peptides C5a and C5a des Arg (the stable serum form) are very potent chemotactants for a wide variety of cells (1). Moreover, C5-derived peptides are considered to be among the most physiologically important chemotactic factors, because they can be generated very rapidly in any vascularized tissue. Consequently, aberrant generation of C5a has been associated with the pathogenesis of numerous inflammatory conditions (1–4). Although C5a has several proinflammatory functions, its role as a chemoattractant is the most prominent. The chemotactic activity of the C5-derived peptides can be enhanced significantly by the vitamin D-binding protein (DBP),3 a plasma protein also known as Gc-globulin (5). Several groups have independently demonstrated this positive chemotactic cofactor function of DBP for C5a/C5a des Arg (i.e., cochemotactic activity) (6–13). The cochemotactic activity of DBP is most readily observed using either suboptimal or nonchemotactic concentrations of the C5-derived peptides (6, 7, 14, 15). However, DBP by itself lacks chemotactic activity, and its chemotactic-enhancing properties appear to be very specific for C5a/C5a des Arg (6–14). Al-
cells with the C5aR (U937-C5aR) provides an excellent cell culture model to investigate C5a-mediated cellular responses (24). U937-C5aR cells express similar levels of cell surface C5aRs as do mature peripheral blood neutrophils (24). Furthermore, the transfectants display a chemotactic dose-response curve to purified C5a (and C5a des Arg) that is identical with that of neutrophils (24). The objective of this study was to determine whether this transfected cell line could be used to investigate the C5a chemotactic cofactor function of DBP in vitro. The results demonstrate that U937-C5aR cells function as a de facto mutant, because they respond only to DBP in serum, but, unlike neutrophils, this cell line does not exhibit C5a chemotactic enhancement either to purified DBP or DBP in plasma. The additional serum factor required for U937-C5aR cells to show enhanced chemotaxis was determined to be platelet-derived thrombospondin-1 (TSP-1).

**Materials and Methods**

**Reagents**

Purified recombinant human C5a was purchased from Sigma-Aldrich (St. Louis, MO). C5a/C5a des Arg RIA was obtained from Amersham Biosciences (Piscataway, NJ). DBP was purified from human plasma and purchased from Bio-Whacked International (Kennebunkport, ME). BSA, ADP, human IgG, and mannan and zymosan A (yeast cell walls from Saccharomyces cerevisiae) were obtained from Sigma-Aldrich. Cobra venom factor (CVF) was purchased from Quidel (San Diego, CA). The protease inhibitors PMSF, 1,10-phenanthroline, and pepstatin A were purchased from Sigma-Aldrich and Pefabloc SC, E-64, and leupeptin were purchased from Roche Applied Science (Indianapolis, IN). Purified TSP-1 and anti-ADP or 2 μM ADP (Sigma-Aldrich) as previously described (25). Brie.

**Isolation of human blood products**

Neutrophils, platelets, serum, and plasma were isolated from the venous blood of healthy, medication-free, paid volunteers who gave informed consent. Neutrophils were purified using a standard three-step isolation procedure described previously (25). Platelets were obtained from blood drawn into syringes containing the anticoagulant acid-citrate-dextrose. Platelet-rich plasma was then obtained by a low-speed centrifugation (150 × g for 15 min), and platelets were isolated subsequently by centrifuging the plasma at 1200 × g for 10 min. Platelets were resuspended in BSA-free Tyrode’s buffer and then stimulated with 100 μM ADP or 2 μM thrombin. Neutrophils were obtained from healthy volunteers by isolation from the peripheral blood by centrifugation (15,000 × g for 2 min) and subsequent washing with buffers containing 0.5% BSA, 0.1% gelatin, 0.15 M NaCl, and 0.1% NaN3.

**DBP immunodepletion of serum and plasma**

Aliquots of serum and plasma (100 μl) first were treated with 50 μl of packed protein G-Sepharose (Amersham Biosciences) to remove human IgG. Samples then were treated for 16 h at 4°C with 1 mg of either a goat polyclonal IgG monospecific against human DBP (DiaSorin, Stillwater, MN) or an irrelevant goat IgG (Sigma-Aldrich). The immune complexes and free goat IgG were removed using protein G-Sepharose (16 h at 4°C on a rocking platform). After the DBP-depleted and sham-depleted material was washed with buffer, the material was resuspended in 20 μl of 2% BSA in HBSS and incubated with 2 μg of a monoclonal anti-human DBP antibody (Becton Dickinson, Hercules, CA). After 30 min on ice, the plasma was passed over a 100 K filter to remove the protein G-Sepharose beads and then frozen at −80°C until need.

**Preparation of complement-activated serum and plasma**

Serum and citrated plasma or DBP-depleted serum and plasma (1 ml each) were incubated for 1 h at 37°C with either 10 mg of zymosan, 167 μl of a 10 mg/ml solution of heat-aggregated human IgG, 10 mg of mannan, or 167 μl of a 0.2 mg/ml solution of CVF. Particulate matter was removed by centrifugation (15,000 × g) for 5 min at 4°C using a microfuge. Samples were then aliquoted and frozen at −80°C.

**In vitro culture of U937 cells**

U937 cells were originally obtained from the American Type Culture Collection (Manassas, VA) and transfected with either the human C5aR or the empty plasmid vector as detailed previously (24). U937 cells were cultured at 37°C in 5% CO2 in RPMI 1640 containing 10% FBS (HyClone, Logan, UT) and 400 μg/ml active G418 (Invitrogen Life Technologies, Carlsbad, CA), and maintained at a density between 2 × 10⁵ and 1.5 × 10⁶ per milliliter.

**SDS-PAGE, IEF, and immunoblotting**

To verify DBP immunodepletion, serum and plasma samples were diluted 1/50 in SDS sample buffer and then separated on SDS-PAGE using an 8–16% gradient polyacrylamide mini-gel (Bio-Rad, Hercules, CA). For qualitative analysis of DBP in serum and plasma, a 1/500 dilution was used. Gels were transferred to polyvinylidene difluoride paper (Millipore, Bedford, MA) and probed using the goat polyclonal anti-human DBP followed by an anti(αb)₂ of rabbit anti-goat IgG linked to alkaline phosphatase (KPL, Gaithersburg, MD). Finally, bands were developed using one-component 5-bromo-4-chloro-3-indolyl phosphate/NBT phosphatase substrate (KPL). DBP in serum and plasma was analyzed for possible structural modifications using IEF. IEF analysis used Servalyt Preparative Polyacrylamide gels (Serva Electrophoresis; purchased from Cresent Chemical, Islandy, NY) that were prefocused for 20 min at 600 V before sample addition. Serum and plasma samples were diluted 1/100 in dH₂O, and 2-μl aliquots were loaded directly onto the gel surface 2 cm from the cathode. Samples were focused for 3.5 h at 1200 V on a cooling platform chilled to 5°C. Focused gels were electroblotted to polyvinylidene difluoride paper for immunoblotting as described above.

**Chemotaxis assay**

Cell movement was quantitated using a 48-well microchemotaxis chamber (Neuroprobe, Cabin John, MD) and 5.0-μm pore size cellulose nitrate filters (purchased from Neuroprobe) as previously described (14). Briefly, cell suspensions and chemotactic factors were prepared and/or diluted in the chemotaxis assay buffer: HBSS supplemented with 10 mM HEPES (pH 7.4) and 1% BSA. Chemotactic factors or assay buffer alone (35 μl) were placed in the lower compartments of the chamber, covered with a 5.0-μm cellulose nitrate filter, and then 50 μl of cell suspensions (4 × 10⁶ neutrophils/ml or 6 × 10⁶ U937 cells/ml) were pipetted into the upper compartments. The chemotaxis chamber was then incubated at 37°C for 30 min (for neutrophils) or 120 min (for U937 cells). After the incubation period, filters were fixed in 2-propanol, stained with acid hematoxylin, cleared with xylenes, and then mounted on microscope slides with Permount (Fisher Scientific, Pittsburgh, PA). Cell movement was quantitated microscopically by measuring the distance in micrometers that the leading front of cells had migrated into the filter according to the method described by Zigmond and Hirsch (26). In each experiment, five fields per duplicate filter were measured at ×400 magnification. The value of the background controls for random neutrophil movement (untreated cells responding to buffer) has been subtracted in all cases so that the neutrophil data are presented as net movement in micrometers per 30 min.

**Data analysis and statistics**

A minimum of three experiments were performed for each assay. Results of several experiments were analyzed for significant differences among group means usingANOVA followed by Newman-Keuls multiple comparisons posttest using the statistical software program InStat (GraphPad Software, San Diego, CA).

**Results**

Because U937-C5aR cells respond to C5a/C5a des Arg in a manner very similar to that of neutrophils, it was reasoned that these cells might be useful for defining the mechanism that permits DBP to function as a unique chemotactic cofactor for C5a. First, we wanted to determine whether DBP enhances C5a-directed chemotaxis in these cells, Fig. 1 shows that neutrophils and U937-C5aR cells exhibit an equivalent chemotactic response to 1 nM C5a and 2.5% zymosan-activated serum (ZAS), both optimal chemotactic concentrations. The negative control of U937 cells transfected with the empty plasmid vector (U937 vector) are unable to respond to either C5a or ZAS. Purified DBP significantly enhanced neutrophil...
movement to a nonchemotactic concentration of C5a (10 pM), whereas U937-C5aR cells fail to respond to this combination (Fig. 1).

In vitro activation of serum complement by zymosan typically generates large quantities of C5a des Arg (1–2 μM), and that level of chemotactic stimulus could mask the cofactor function of DBP (prominently observed at suboptimal or nonchemotactic levels of the C5-derived peptides). To examine more thoroughly whether DBP can function as a chemotactic cofactor to a defined nonchemotactic concentration of C5a (10 pM), various sources of DBP were tested to determine whether they could augment U937-C5aR cell movement. Purified DBP (obtained from pooled human plasma) as well as 1% dilutions of heparinized plasma, citrated plasma, citrated plasma that was clotted by the addition of Ca2+ and glass beads (clotted plasma), or serum were used as sources of DBP (Fig. 2). The 1% dilutions of serum and plasma by themselves were not chemotactic for either neutrophils or U937-C5aR cells (data not shown). Furthermore, a 1% dilution contains an equivalent amount of DBP as used for the purified protein (50 nM).

Serum and plasma samples also were pretreated with Plummer’s inhibitor (1 mM; Calbiochem, San Diego, CA) to abolish the activity of carboxypeptidase N (anaphylatoxin inactivator) and prevent conversion of C5a to C5a des Arg. It is evident from the data presented in Fig. 2 that neutrophils show chemotactic movement to any DBP source, whereas U937-C5aR cells only respond to DBP in serum derived from clotted whole blood. The information contained in Figs. 1 and 2 highlight the fact that mature peripheral blood neutrophils respond to DBP differently from undifferentiated U937 cells, but also may suggest that DBP is modified to an active chemotactic form in serum, and this can be detected using the U937-C5aR cells.

There are numerous potential modifications to DBP in serum that may permit the protein to function as a chemotactic cofactor for C5a. The most likely extracellular modifications would be proteolysis or deglycosylation, yet we consistently observe no alteration in the molecular mass of DBP in serum vs plasma by SDS-PAGE and immunoblotting (Fig. 3A). However, because small...
The contribution of DBP to the chemotactic activity of complement-activated serum and plasma next was investigated using DBP-depleted samples. Aliquots of serum or citrated plasma were treated with polyclonal goat anti-human DBP, or sham-treated with an irrelevant goat IgG, and immune complexes were removed as described in Materials and Methods. Fig. 4A shows that the immunodepletion protocol was effective in removing DBP from serum and plasma (note: gel was overloaded with a 10-fold greater amount of sample than typically used to detect DBP by immunoblotting). Next, DBP-depleted or sham-treated samples were activated with zymosan, and cell movement to ZAS and ZAP was compared. Fig. 4B demonstrates that neutrophils respond equally well to either sham-treated ZAS or ZAP; however, U937-C5aR cell movement to ZAP was ~50% that of ZAS. This was a very consistent finding among several preparations of ZAS and ZAP, despite the fact that they contained equivalent amounts of C5a/C5a des Arg as measured by RIA (1.82 ± 0.19 μg/ml for ZAS; 1.86 ± 0.20 μg/ml for ZAP; both n = 7). Fig. 4B also revealed that DBP depletion of serum or plasma significantly reduced the chemotactic activity of ZAS and ZAP even though C5a/C5a des Arg levels essentially were unaltered (1.75 μg/ml after DBP depletion), indicating that DBP is required for maximal chemotactic activity. Furthermore, activation of complement in serum and plasma with either CVF (alternative pathway), aggregated human IgG (classical pathway), or mannan (mannan-binding lectin pathway) produced results (data not shown) identical with that with zymosan (Fig. 4B), indicating that the mode of complement activation does not alter the chemotactic function of DBP.

Because U937-C5aR cells can detect a difference in the C5a cofactor function of DBP in ZAS vs ZAP, we next chose to determine whether the chemotactic activity of ZAP could be augmented by supplementing with either serum or activated platelet releasates. Fig. 5 shows that supplementation of ZAP with either serum or activated platelet releasate enhances its chemotactic activity to the level of ZAS. The chemotactic activity of ZAS supplemented with serum or platelet releasates was not altered (Fig. 5). Interestingly, serum depleted of DBP also could enhance the chemotactic activity of ZAP (Fig. 5), suggesting that, in addition to DBP, products released into serum during the clotting process are required for maximal chemotactic movement of U937-C5aR cells to C5a/C5a des Arg. Therefore, to investigate whether products released from activated platelets can permit DBP to function as a C5a cofactor for U937-C5aR cells, purified DBP was added to platelet releasate and its capacity to enhance chemotaxis to 10 pM C5a was measured. Fig. 6 shows that platelet products allow purified DBP to function as a chemotactic cofactor. This activity in platelets is not proteolytic because platelet releasate treated with a protease inhibitor mixture (Plts PI) was equally effective in permitting DBP to function as a C5a chemotaxin (Fig. 6). The data presented in Figs. 5 and 6 indicate that factors released by platelets into serum during the clotting process function as an additional cofactor for DBP.

Finally, activated platelet releasate was fractionated by gel filtration fast protein liquid chromatography using Superdex 200 column (Amersham Biosciences) to identify the platelet-derived factor. A fraction with a molecular mass range from 400 to 600 kDa that could enhance the chemotactic activity of ZAP to the level of ZAS was identified (data not shown). This fraction also contained a large percentage of the total protein in the platelet releasate. Because TSP-1 has a molecular mass of 450 kDa and is a major protein released by activated platelets, we investigated its role by using purified TSP-1 and a specific Ab against TSP-1. Fig. 7 demonstrates that purified TSP-1 mimics the effect of serum or platelet releasate on enhancing the chemotactic activity of ZAP. In addition, treatment of serum or platelet releasate with anti-TSP-1 eliminates their ability to enhance the chemotactic activity of ZAP. These results demonstrate that platelet-derived TSP-1 is necessary for DBP to function as a chemotactic cofactor for C5a.
responses (34). TSP-1 mediates its cellular effects via numerous additional chemotactic cofactor for C5a. This study clearly shows that it functions along with DBP as an integrins (VIIIb/H9251). Undifferentiated U937 cells do not express TSP-1 with respect to DBP-mediated chemotaxis enhancement to C5a. Results also show that DBP is absolutely essential for maximal chemotaxis to complement-activated serum, because DBP depletion decreases the chemotactic activity by almost 75% without altering levels of TSP-1, as measured by a commercially available ELISA (Chemicon, Temecula, CA), so the 1% dilution of supplemented ZAP was significantly greater (p < 0.001) than to control ZAP. In addition, cells moved significantly less (p < 0.001) to control ZAP vs ZAS.

Discussion

The results of this study demonstrate that platelet-derived TSP-1 functions with DBP to enhance the chemotactic activity of C5a. This novel finding was made possible because U937-C5aR cells function as a de facto mutant (compared with neutrophils) with respect to DBP-mediated chemotaxis enhancement to C5a. Results also show that DBP is absolutely essential for maximal chemotaxis to complement-activated serum, because DBP depletion decreases the chemotactic activity by almost 75% without altering levels of C5a (Fig. 4B). Undifferentiated U937 cells do not express TSP-1 (28), and hence show enhanced C5a chemotaxis only in the presence of serum (DBP plus TSP-1). In contrast, mature neutrophils display enhanced C5a movement to any source of DBP (purified protein, plasma, or serum), and these cells have been reported to express cell surface TSP-1 to varying degrees (39). We have noted for many years that neutrophils need to be activated to some extent to bind DBP and show a cochemotactic response to C5a. Neutrophils isolated from blood using minimal manipulation and the most stringent endotoxin-free conditions display both a lack of DBP binding and cochemotactic response to C5a (R. R. Kew, unpublished observations). This may explain why it takes 15–20 min after DBP is added to neutrophils before cochemotactic activity is detected (14, 15). However, from a physiological perspective, this makes sense, because one would want cochemotactic movement to C5a only after leukocytes have exited the vasculature.

DBP appears to bind to many of the same “scavenger-type” receptors as TSP-1, such as cell surface proteoglycans (16), LDL receptor family members (18, 19), and possibly CD36 (20). Furthermore, the deglycosylated macrophage-activating form of DBP can mimic the antiangiogenic effects of TSP-1 on endothelial cells (20), suggesting that DBP and TSP-1 may compete for the same receptors. Numerous reports have demonstrated that TSP-1 can induce chemotaxis of several cell types (42–44). High concentrations of TSP-1 (300–1000 nM) have been shown to induce chemotaxis of neutrophils, monocytes, and HL-60 cells (42–44), whereas low concentrations of TSP-1 (30–50 nM) can prime neutrophils to enhance their chemotaxis to formyl peptides (42). However, in this study, the concentration of TSP-1 used was considerably lower than those described above. TSP-1 concentrations in human serum can vary widely, but the pooled serum we used contained 36 μg/ml (~80 nM), as measured by a commercially available ELISA (Chemicon, Temecula, CA), so the 1% dilution of serum used in all experiments contained 0.8 nM TSP-1. The level of purified TSP-1 added to ZAP was 2.2 nM (Fig. 7); thus, both of these TSP-1 concentrations are far below the chemotactic or priming levels reported previously. It is not clear how DBP and TSP-1 mediate chemotaxis enhancement to C5a, but it is reasonable to infer that it may involve bridging of signaling molecules on the plasma membrane. Indeed, determining how DBP and TSP-1 interact with cell surface TSP-1 receptors is an ongoing investigation in our laboratory.

The physiological significance of DBP as a chemotactic cofactor for C5a/C5a des Arg is not widely appreciated. Although the initial descriptions of a C5a chemotactic factor in serum were reported >25 years ago (45–47), and DBP was subsequently identified as the serum-derived chemotactic cofactor (6, 7), the mechanism by which DBP enhances chemotaxis to C5a still is not known. The most obvious explanation for its chemotactic effect would be an alteration of C5aR number and/or Kd for C5a. However, this possibility largely has been discounted, because addition of DBP to neutrophils does not alter these properties of the C5aR (10, 48).
FIGURE 7. Effect of TSP-1 on the chemotactic activity of ZAS and ZAP for U937-C5aR cells. Purified TSP-1 (10 µg/ml) or 50 µg/ml polyclonal anti-TSP-1 was added to 1% dilutions of ZAS and ZAP. U937-C5aR cells (6 × 10^6/ml) in chemotaxis buffer were allowed to respond to the samples for 120 min at 37°C. Numbers represent mean ± SEM of three experiments. Asterisks denote that cell movement to indicated samples was significantly different (p < 0.001) than the corresponding control value.

Furthermore, DBP does not appear to interact directly with the C5aR on the cell surface, because we have never observed DBP-C5aR complexes by either coimmunoprecipitation or chemical cross-linking (R. R. Kew, unpublished observations). Any model to explain the chemotactic cofactor function of DBP must take into account two key observations: first, DBP enhances chemotaxis only to C5a/C5a des Arg, and second, DBP does not alter C5a-mediated antimicrobial functions in neutrophils (oxidant generation, degranulation) (6–13). These facts imply specificity in the DBP-mediated signal and exclusive coupling to the signaling pathways that control cell movement. Previous reports from this laboratory have demonstrated that DBP chemotactic activity is generated on the neutrophil surface (independent of C5a binding to its receptor) within 15–20 min after binding, suggesting that a signal requires time to be processed or components of a binding site complex need to be assembled (14, 15). DBP binds to chondroitin sulfate proteoglycans on the neutrophil plasma membrane, and its binding is regulated by cell surface elastase (16, 17). In addition, DBP binding to proteoglycans on neutrophil plasma membrane cause oligomerization and clustering of the protein (16). Therefore, we propose that DBP functions with TSP-1 as a cell surface adaptor molecule to induce clustering of receptors and/or signaling components. Nevertheless, given our current level of understanding, any proposed mechanism to explain the C5a chemotactic cofactor effect of DBP is tentative.

It has been suggested for many years that C5a is a paramount chemoattractant in vivo (49, 50). More recent in vitro evidence has further strengthened this concept, demonstrating that C5a functions as a primary end-target chemoattractant (51, 52). Therefore, cofactors that significantly enhance the chemotactic activity of C5a, such as DBP, also would be very important physiologically. DBP is a ubiquitous protein in vivo; it has been detected in almost all body fluids at levels capable of inducing chemotaxis to C5a (53). Moreover, no homozygous deficiency of DBP has been reported in any mammal, although a viable DBP−/− mouse has been generated (54). Thus, DBP would be present any time C5a is produced and would be available to mediate its chemotactic effect. In addition, platelet-derived TSP-1 probably also would be present at a site of inflammation where complement has been activated. This paper demonstrates for the first time that TSP-1 acts with DBP to enhance C5a-mediated chemotaxis. In addition, results show that the transfected cell line U937-C5aR will be very useful for dissecting the chemotactic mechanisms of DBP in vitro.

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