Published in final edited form as:

Mol Immunol. 2006 March ; 43(8): 1109–1115.

Selective Inhibition of the C5a Chemotactic Cofactor Function of the Vitamin D Binding Protein by 1,25(OH)₂Vitamin D₃

Anisha B. Shah, Stephen J. DiMartino, Glenda Trujillo, and Richard R. Kew

Department of Pathology, Stony Brook University School of Medicine, Stony Brook, NY 11794-8691

Abstract

The vitamin D binding protein (DBP) is a multifunctional plasma protein that can significantly enhance the chemotactic response to complement fragment C5a. The chemotactic cofactor function of DBP requires cell surface binding in order to mediate this process. The goal of this study was to investigate the effect of ligating DBP with its two primary physiological ligands, vitamin D and G-actin, on both binding to neutrophils and the ability to enhance chemotaxis to C5a. There was no difference in neutrophil binding between of the holo (bound) forms versus the apo (unbound) form of radioiodinated DBP, indicating that the cell binding region of DBP likely is distinct from the vitamin D sterol and G-actin binding sites. Likewise, G-actin, 25(OH)D₃, and G-actin plus 25(OH)D₃ bound to DBP did not alter its capacity to enhance chemotaxis toward C5a. However, the active form of vitamin D (1,25(OH)₂D₃) completely eliminated the chemotactic cofactor function of DBP. Dose response curves demonstrated that as little as 1 pM 1,25(OH)₂D₃ significantly inhibited chemotaxis enhancement. Moreover, at physiological concentrations 1,25(OH)₂D₃ needs to be bound to DBP to mediate the inhibitory effect. Neutrophil chemotaxis to optimal concentrations of C5a, formyl peptide, CXCL8 or leukotriene B₄ was not altered by 1,25(OH)₂D₃ indicating that the active vitamin does not have a global inhibitory effect on neutrophil chemotaxis. Finally, inhibition of cell surface alkaline phosphatase with sodium orthovanadate completely reversed the inhibitory effect of 1,25(OH)₂D₃. These results indicate that the cell binding and co-chemotactic functions of DBP are not altered when the protein binds G-actin and/or vitamin D. Furthermore, the co-chemotactic signal from DBP can be eliminated or counteracted by 1,25(OH)₂D₃.

Keywords

Complement; Chemotaxis; Neutrophils; Inflammation; Vitamin D

INTRODUCTION

Leukocytes are recruited to sites of inflammation by numerous chemoattractants, but one of the most potent and early-acting chemotactic factors is complement activation peptide C5a (and its stable serum form C5a des Arg) (Guo and Ward, 2005). The chemotactic activity of C5a and C5a des Arg can be enhanced significantly by the vitamin D binding protein (DBP), a plasma protein also known as Gc-globulin (Binder et al., 1999; Kew and Webster, 1988; Metcalf et al., 1991; Perez et al., 1988; Zwahlen and Roth, 1990). DBP is a multifunctional and highly polymorphic plasma protein synthesized primarily in the liver, has a molecular mass of approximately 56 kDa and circulates in plasma at 6 to 7 μM (Gomme and Bertolini, 2004; White and Cooke, 2000). DBP is a member of the albumin (*ALB*), α-fetoprotein (*AFP*), and α-albumin/afamin (*AFM*) gene family and has the characteristic multiple disulfide-bonded, triple domain modular structure (White and Cooke, 2000). Besides functioning as a circulating

vitamin D transport protein, it has been demonstrated that plasma DBP effectively scavenges G-actin released at sites of necrotic cell death and prevents polymerization of actin in the circulation (Gomme and Bertolini, 2004; White and Cooke, 2000). Distinct binding regions within the 458 amino acid sequence of DBP have been identified. Analysis of the crystal structure of DBP (bound to either vitamin D₃ or actin) has confirmed previous studies that the vitamin D sterol binding segment resides in the N-terminal domain (amino acids 35–49) (Haddad et al., 1992; Swamy et al., 1997), and also revealed that actin interacts with distinct amino acid sequences in all three DBP domains (Head et al., 2002; Otterbein et al., 2002; Swamy et al., 2002; Verboven et al., 2002). Recently, our lab has identified a C5a chemotactic cofactor region in the N-terminal domain (amino acids 130–149), a sequence distinct from either the vitamin D or G-actin binding regions (Zhang and Kew, 2004).

The precise mechanisms by which DBP acts as a chemotactic cofactor for C5a (co-chemotactic activity) are not known. However, previous studies have shown that neutrophils transiently generate co-chemotactic activity for C5a/C5a des Arg on the cell surface within 15–20 min of DBP binding (Kew et al., 1995a). These cells also utilize membrane-bound elastase to shed the DBP-binding site into the extracellular milieu (DiMartino et al., 2001). Both plasma membrane binding and subsequent shedding of DBP are essential for the protein to function as a chemotactic cofactor for C5a. Recent studies from our lab also have shown that DBP requires platelet-derived thrombospondin-1 for maximal co-chemotactic activity (Trujillo and Kew, 2004), and that cell surface CD44 and annexin A2 mediate the C5a chemotactic cofactor function of DBP (McVoy and Kew, 2005). Nevertheless, a fundamental question that remains is what effect does ligation of DBP with its primary physiological ligands, vitamin D and G-actin, have on the C5a chemotactic cofactor activity. The results demonstrate that DBP bound to G-actin, 25(OH)D₃, or G-actin plus 25(OH)D₃ did not alter either its binding to neutrophils or its capacity to enhance chemotaxis toward C5a. However, the active form of vitamin D (1,25(OH)₂D₃) bound to DBP completely eliminated the C5a chemotactic cofactor function and this inhibition requires cell surface alkaline phosphatase activity.

MATERIALS AND METHODS

Reagents

Purified human vitamin D binding protein (DBP) was purchased from Biodesign International (Kennebunkport, ME). Purified recombinant human C5a, formyl norleucyl-leucyl-phenylalanine (fNLP), leukotriene B₄ (LTB₄) and zymosan A (yeast cell walls from *S. cerevisiae*) were purchased from Sigma-Aldrich (St. Louis, MO). Complement-activated serum was generated by incubating 1 ml of human serum with 10 mg zymosan A for 1 h at 37°C. Particulate matter was removed by centrifugation (15,000 x g) and samples were aliquoted and frozen at –80°C. Recombinant CXCL8 (IL-8) was obtained from R&D Systems (Minneapolis, MN). The 25(OH) and 1,25(OH)₂ forms of vitamin D₃ were purchased from BioMol (Plymouth Meeting, PA). G-actin was purified from rabbit skeletal muscle as previously described (Spudich and Watt, 1971).

Isolation of Human Neutrophils

Neutrophils, serum, and plasma were isolated from the venous blood of healthy, medication-free, paid volunteers who gave informed consent. The Institutional Review Board of Stony Brook University approved this procedure. These procedures have been described in detail previously (Kew et al., 1995a).

Quantitative Binding of Radioiodinated DBP to Neutrophils

Purified DBP (200–400 µg) was iodinated using Na-¹²⁵I (Dupont-NEN, Wilmington, DE) as previously described (DiMartino and Kew, 1999). Neutrophils (10⁷ cells/sample) were

incubated with 100 nM ^{125}I -DBP in HBSS containing 0.1% BSA (assay buffer) in a total volume of 100 μl . Samples were incubated at 37°C for 60 min after which cells were placed on ice and then washed twice with ice-cold assay buffer (1.0 ml each) and centrifuged for 7 min at 200 x g at 2°C. The cell pellets then were counted for total radioactivity in a gamma counter. All samples were assayed in triplicate or quadruplicate.

Neutrophil 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 (Kew et al., 1995a). Cell suspensions and chemotactic factors were prepared and/or diluted in the chemotaxis assay buffer (Hank's balanced salt solution (HBSS) supplemented with 10 mM HEPES (pH 7.4) and 1% bovine serum albumin, BSA). Cell movement was quantitated microscopically by measuring the distance in microns (μm) that the leading front of cells had migrated into the filter according to the method described by Zigmond and Hirsch (Zigmond and Hirsch, 1973). In each experiment, five fields per duplicate filter were measured at 400 X magnification. The value of the background controls (untreated cells responding to buffer) has been subtracted in all cases so that the data are presented as net neutrophil (PMN) movement in $\mu\text{m}/\text{time}$ of incubation. The mean migration distance of the untreated buffer control from all experiments was $45 \pm 3.4 \mu\text{m}/30 \text{ min}$ ($n = 24$).

Neutrophil Alkaline Phosphatase Assay

Alkaline phosphatase activity on the plasma membrane of viable neutrophils was measured using the fluorescent substrate 6,8-difluoro-4-methylumbelliferyl phosphate (DiFMUP) and the EnzChek Phosphatase Assay Kit (Molecular Probes, Eugene, OR). Neutrophils (5×10^6 cells) were suspended in 50 μl of 0.1 M sodium acetate (pH 5.0) and were immediately added to microtiter plates containing 50 μl of 0.2 mM DiFMUP substrate and incubated for 5 min at 22°C in the dark. A standard curve was generated using known amounts of the fluorescent compound 6,8-difluoro-7-hydroxy-4-methylcoumarin. Fluorescence was measured at 358 nm excitation and 455 nm emission using a SpectraMax M2 microtiter plate reader (Molecular Devices, Sunnydale, CA).

Data Analysis and Statistics

A minimum of 3 experiments was performed for each assay, using neutrophils from different individuals. Results from several experiments were analyzed for significant differences among group means using the Newman-Keul's Multiple Comparisons test utilizing a statistical software program InStat (GraphPad Software, San Diego, CA).

RESULTS

Previous work from our laboratory has demonstrated that the binding of DBP to neutrophils is essential for the chemotaxis enhancement of C5a (Kew et al., 1995a; Kew et al., 1995b). More recent studies have shown that the region of DBP that mediates co-chemotactic activity resides in its N-terminal domain, distinct from the vitamin D sterol or G-actin binding regions (Zhang and Kew, 2004). Therefore, to determine if ligation of DBP with either vitamin D and/or G-actin alters the ability to bind to neutrophils, the binding of apo (unligated) versus holo forms of radioiodinated DBP to neutrophils was measured. The binding of G-actin to ^{125}I -DBP was confirmed by complex formation on gel filtration chromatography, the binding of vitamin D was confirmed by measuring the inhibition of ^3H -vitamin D binding to DBP in the presence of unlabeled competitor vitamin (data not shown). Figure 1A clearly shows that saturation of DBP with vitamin D, G-actin or both ligands does not alter its ability to bind to neutrophils, suggesting that the cellular binding region on DBP is distinct from either the vitamin D sterol or G-actin binding sites. Since cell binding is prerequisite for C5a co-chemotaxis, the co

chemotactic activity of apo versus holo forms of DBP was measured. Figure 1B demonstrates that G-actin and the major plasma form of vitamin D ($25(\text{OH})\text{D}_3$), bound to DBP either individually or together, did not alter its ability to enhance neutrophil chemotaxis toward C5a. However, the hormonally-active form of vitamin D ($1,25(\text{OH})_2\text{D}_3$) bound to DBP completely eliminated the co-chemotactic effect, despite the fact that this form of the vitamin did not alter DBP binding to neutrophils (compare Figs. 1A and 1B). The concentration of $1,25(\text{OH})_2\text{D}_3$ used in Figure 1 (100 nM) is about 1000-fold greater than the physiological plasma concentration of the active vitamin. Therefore, to determine the effect of various concentrations of vitamin D on the co-chemotactic activity of DBP, dose response curves were generated. Figure 2 shows that $25(\text{OH})\text{D}_3$ bound to DBP had no effect on co-chemotactic activity over a wide concentration range (10^{-6} to 10^{-13} M). In contrast, $1,25(\text{OH})_2\text{D}_3$ bound to DBP completely inhibited its C5a co-chemotactic activity from 1 μM (10^{-6} M) to 10 pM (10^{-11} M), and there was significant inhibition at 1 pM (Fig. 2). These inhibitory levels of $1,25(\text{OH})_2\text{D}_3$ are well within the physiological concentration range (40 to 100 pM). These results indicate that only the active form of vitamin D ($1,25(\text{OH})_2\text{D}_3$) inhibits the co-chemotactic effect of DBP.

The previous experiments demonstrated that $1,25(\text{OH})_2\text{D}_3$ bound to DBP was a potent inhibitor of C5a co-chemotaxis (Figs. 1B & 2). Therefore, the next question we addressed was does $1,25(\text{OH})_2\text{D}_3$ need to be bound to DBP to have an inhibitory effect. For these experiments we exploited the fact that $25(\text{OH})\text{D}_3$ binds to DBP with a 10-fold tighter affinity than does $1,25(\text{OH})_2\text{D}_3$ (Cooke and Haddad, 1989), and that the $25(\text{OH})\text{D}_3$ form of the vitamin does not alter the co-chemotactic activity of DBP (Figs. 1B & 2). Figure 3 shows that, at a physiological concentration of 100 pM, $1,25(\text{OH})_2\text{D}_3$ needs to be bound to DBP to inhibit co-chemotaxis. However, very high concentrations of free $1,25(\text{OH})_2\text{D}_3$ (> 10 nM) could inhibit the co-chemotactic function of DBP (data not shown). Next, the effect of $1,25(\text{OH})_2\text{D}_3$ on neutrophil movement toward other chemotactic stimulus was examined. Figure 4 demonstrates that 10 nM $1,25(\text{OH})_2\text{D}_3$ bound to DBP does not alter neutrophil chemotaxis to optimal concentrations of C5a, formyl peptide, LTB_4 or CXCL8 (IL-8) indicating that even super physiological concentrations of the active vitamin do not alter the chemotactic capacity of neutrophils. These results indicate that $1,25(\text{OH})_2\text{D}_3$ selectively inhibits the co-chemotactic activity of DBP.

Finally, the possible mechanism of the selective inhibition of DBP co-chemotactic activity by $1,25(\text{OH})_2\text{D}_3$ was investigated. Neutrophils express abundant quantities of the enzyme alkaline phosphatase (AP) on their plasma membranes (Borregaard et al., 1995) and $1,25(\text{OH})_2\text{D}_3$ has been shown to increase the activity of this enzyme in several non-myeloid cell types (Gill and Bell, 2000; Mulkins et al., 1983; Schwartz et al., 1991). Furthermore, a plasma membrane form of the vitamin D receptor (VDR) that mediates rapid signaling events has been described recently and this receptor associates with annexin A2 in lipid rafts (Huhtakangas et al., 2004; Mizwicki et al., 2004). AP on the external face of the plasma membrane also has been shown to associate with annexin A2 in lipid rafts (Gillette and Nielsen-Preiss, 2004). Moreover, our lab has recently shown that annexin A2 may serve as part of the DBP cell surface binding site (McVoy and Kew, 2005). These studies have provided the rationale to investigate if there is an association between $1,25(\text{OH})_2\text{D}_3$ and AP. Therefore, the effect of the $1,25(\text{OH})_2\text{D}_3$ and the phosphatase inhibitor sodium orthovanadate (SOV) on neutrophil AP activity and co-chemotactic activity was examined. AP activity was measured on viable purified neutrophils (5×10^6 cells) using the fluorescent substrate DiFMUP. As expected neutrophils contained abundant AP activity ($11,107 \pm 1335$ units), however, enzyme activity was not significantly altered by pretreating cells with 10 nM $1,25(\text{OH})_2\text{D}_3$. In contrast, 10 μM SOV almost completely inhibited neutrophil AP activity (667 ± 111 units) but did not alter cell viability (data not shown). Figure 5 demonstrates that pretreating neutrophils with 10 μM SOV also completely reverses the inhibitory effect of $1,25(\text{OH})_2\text{D}_3$ on the C5a co-chemotactic activity of DBP, while SOV alone has no effect on the cells ability to migrate towards C5a plus DBP.

This data may suggest that $1,25(\text{OH})_2\text{D}_3$ requires AP activity to inhibit the co-chemotactic function of DBP.

DISCUSSION

This paper reports three major findings: (1) cell surface binding and co-chemotactic activity of DBP are not affected when bound to G-actin, vitamin D or both ligands; (2) the hormonally active form of vitamin D ($1,25(\text{OH})_2\text{D}_3$), when bound to DBP, completely inhibits co-chemotactic activity; (3) $1,25(\text{OH})_2\text{D}_3$ may utilize cell surface alkaline phosphatase activity to inhibit the chemotactic cofactor function. The first conclusion provides a functional correlate to our recent study that identified a C5a chemotactic cofactor region in the N-terminal domain of DBP (amino acids 130–149), a sequence distinct from either the vitamin D or G-actin binding regions (Zhang and Kew, 2004). The second and third findings suggest that DBP may induce a co-stimulatory signal that synergizes with the primary signal of C5a binding to the C5a receptor. The inhibitory effect of $1,25(\text{OH})_2\text{D}_3$ on the co-chemotactic activity of DBP is important evidence to indicate that the chemotactic cofactor effect of DBP can be uncoupled from the primary chemotactic signal induced by C5a. Moreover, inhibition of cell surface alkaline phosphatase reverses this negative regulatory effect of $1,25(\text{OH})_2\text{D}_3$. This information will be essential for understanding the precise mechanism of C5a chemotaxis enhancement by DBP. Although the majority of the results in this study are novel, a survey of the literature revealed that a brief communication previously reported that 1 nM $1,25(\text{OH})_2$ inhibited the co-chemotactic activity of purified DBP (Petrini et al., 1991), however, no other information was provided. The current study presents a more comprehensive investigation of the effect of $1,25(\text{OH})_2\text{D}_3$ on the C5a chemotactic cofactor function of DBP.

The action of $1,25(\text{OH})_2\text{D}_3$ on neutrophils is likely mediated via a cell surface vitamin D receptor (VDR). The classic VDR is a nuclear receptor found in a wide variety of cells that mediates genomic effects (Brown et al., 1999). In contrast, a plasma membrane form of the VDR recently has been described that is thought to be responsible for mediating the rapid signaling effects of $1,25(\text{OH})_2\text{D}_3$ (Huhtakangas et al., 2004; Mizwicki et al., 2004). However, it is not known if the cell surface VDR is the same as the nuclear receptor or is a distinct plasma membrane protein. Furthermore, the plasma membrane form of the VDR associates with annexin A2 in lipid rafts (Huhtakangas et al., 2004; Mizwicki et al., 2004), a unique membrane microenvironment for assembly of signaling complexes (Brown and London, 1998; Gomez-Mouton et al., 2004). Annexin A2 is a widely expressed member of a family of Ca^{2+} -dependent phospholipid binding proteins that has been shown to facilitate formation of signaling complexes on both the external and cytoplasmic faces of the plasma membrane (Merrifield et al., 2001). Our lab has recently shown that annexin A2 serves as part of the DBP cell surface binding site (McVoy and Kew, 2005). Interestingly, AP on the external face of the plasma membrane also has been shown to associate with annexin A2 in lipid rafts (Gillette and Nielsen-Preiss, 2004). Therefore, the putative cell surface DBP binding site complex may include both the VDR and AP. It is reasonable to speculate that a cell surface form of the VDR would be part of a binding site complex for the vitamin D sterol carrier protein DBP, since this would facilitate efficient delivery of vitamin D to cells. However, it is not clear why AP would be associated with the DBP binding site. Since AP activity is needed to regulate the co-chemotactic activity of DBP (Fig. 5) perhaps the enzyme functions by dephosphorylating a protein(s) substrate on the cell surface. In this way AP may terminate the action of a functional DBP binding/signaling complex. Nevertheless, given our current level of understanding, any proposed model to explain the inhibitory actions of $1,25(\text{OH})_2\text{D}_3$ and AP on the C5a chemotactic cofactor function of DBP is tentative.

It is interesting to note that the concentrations of $1,25(\text{OH})_2\text{D}_3$ that inhibit the co-chemotactic activity of DBP span the physiological plasma range of $1,25(\text{OH})_2\text{D}_3$ (from 40 to 100 pM, with

a mean plasma concentration of about 60 pM). Therefore, in vivo concentrations of active vitamin D should prevent DBP from functioning as a chemotactic cofactor for C5a. However, 5–10% complement-activated serum is a widely utilized and very potent leukocyte chemoattractant. This amount of serum would contain approximately 3–6 pM 1,25(OH)₂D₃, a concentration that should inhibit the co-chemotactic response (see Fig. 2) but obviously does not. Moreover, the chemotactic activity in complement-activated serum is DBP-dependent because immunodepletion of DBP from activated serum reduces the chemotactic activity by almost 75% without altering the levels of C5a (Kew and Webster, 1988; Trujillo and Kew, 2004). This apparent paradox may be explained by how and when 1,25(OH)₂D₃ interacts with neutrophils. In the present study, cells were pretreated with either the unligated apo form of DBP, or the protein containing bound ligands, before adding cells to the chemotaxis chamber for migration to C5a. Consequently, 1,25(OH)₂D₃ would have sufficient time to interact with the cell surface VDR and transmit an inhibitory signal. In contrast, neutrophils migrating toward 5% complement-activated serum would have initiated a chemotactic response either prior or concurrently with DBP (plus vitamin D) binding to the cell, so 1,25(OH)₂D₃ would not have adequate time to inhibit the co-chemotactic response. In addition, the low levels of 1,25(OH)₂D₃ in dilute serum (< 10 pM) may be inadequate to inhibit the chemotactic cofactor function in a very complex mixture of molecules, i.e., serum. Perhaps the physiological concentrations of 1,25(OH)₂D₃ (40 to 100 pM) found in blood inhibit the neutrophil co-chemotactic response to C5a, whereas lower concentrations (10 to 50-fold lower DBP levels) found in extracellular tissue spaces permits DBP to enhance C5a chemotaxis. This would make sense since immune mechanisms are generally not meant to function in the blood and are tightly regulated until cells exit the vasculature.

Enhancement of complement-dependent chemotactic activity by DBP has been observed in several cell types (Kew and Webster, 1988; Metcalf et al., 1991; Perez et al., 1988; Petrini et al., 1991; Piquette et al., 1994; Robbins and Hamel, 1990; Senior et al., 1988; Zwahlen and Roth, 1990). This phenomenon almost certainly has physiological significance since DBP appears to be ubiquitous in the body (Cooke and Haddad, 1989). It is found at high concentrations in blood (6–7 μM) and has been detected in several other fluids including cerebrospinal fluid, bronchoalveolar lining fluid, seminal fluid, saliva, synovial fluid and breast milk. However, very little mechanistic information is available concerning how DBP enhances chemotaxis to the C5 derived peptides. Several studies from our laboratory have demonstrated that the binding of DBP to the neutrophil plasma membrane, and subsequent protease-mediated shedding of the binding site, are essential for the chemotaxis enhancement of C5a (DiMartino and Kew, 1999; DiMartino et al., 2001; Kew et al., 1995a; Kew et al., 1995b). More recently, we have demonstrated that DBP requires platelet-derived thrombospondin-1 for maximal co-chemotactic activity (Trujillo and Kew, 2004). In addition, cell surface CD44 and annexin A2 are part of a DBP binding site complex and mediate the C5a chemotactic cofactor function of DBP (McVoy and Kew, 2005). The current study provides evidence that the putative cellular binding site on DBP is not altered by ligation with vitamin D sterols or G-actin. In addition, 1,25(OH)₂ vitamin D₃ bound to DBP specifically inhibits C5a co-chemotactic activity and the active vitamin may utilize cell surface AP activity to mediate the inhibition. We believe that this new information will provide important insights into how DBP specifically enhances the chemotactic activity of C5a.

Acknowledgements

This investigation was supported in part by National Institutes of Health grant GM 63769 (to R.R.K.). A.B.S. was supported in part by the M.D. with Recognition in Research Program, Stony Brook University School of Medicine. S.J.D. was supported in part by a Medical Scientist Training Program (MSTP) grant from the National Institutes of Health. G.T. was supported by a W. Burghardt Turner Fellowship and the National Science Foundation-funded AGEP Program (both at Stony Brook University), and by a training grant GM 08468 from the National Institutes of Health.

References

- Binder R, Kress A, Kan G, Herrmann K, Kirschfink M. Neutrophil priming by cytokines and vitamin D binding protein (Gc-globulin): impact on C5a-mediated chemotaxis, degranulation and respiratory burst. *Mol Immunol* 1999;36:885–892. [PubMed: 10698343]
- Borregaard N, Kjeldsen L, Lollike K, Sengelov H. Granules and secretory vesicles of the human neutrophil. *Clin Exp Immunol* 1995;101:6–9. [PubMed: 7606861]
- Brown AJ, Dusso A, Slatopolsky E. Vitamin D. *Am. J Physiol* 1999;277:F157–175.
- Brown DA, London E. Functions of lipid rafts in biological membranes. *Annu Rev Cell Dev Biol* 1998;14:111–136. [PubMed: 9891780]
- Cooke NE, Haddad JG. Vitamin D binding protein (Gc-globulin). *Endocr Rev* 1989;10:294–307. [PubMed: 2476303]
- DiMartino SJ, Kew RR. Initial characterization of the vitamin D binding protein (Gc-globulin) binding site on the neutrophil plasma membrane. Evidence for a chondroitin sulfate proteoglycan. *J Immunol* 1999;163:2135–2142. [PubMed: 10438954]
- DiMartino SJ, Shah AB, Trujillo G, Kew RR. 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. *J Immunol* 2001;166:2688–2694. [PubMed: 11160333]
- Gill RK, Bell NH. Steroid receptor co-activator-1 mediates 1,25-dihydroxyvitamin D(3)-stimulated alkaline phosphatase in human osteosarcoma cells. *Calcified Tissue Int* 2000;66:370–374.
- Gillette JM, Nielsen-Preiss SM. The role of annexin 2 in osteoblastic mineralization. *J Cell Sci* 2004;117:441–449. [PubMed: 14679310]
- Gomez-Mouton C, Lacalle RA, Mira E, Jimenez-Baranda S, Barber DF, Carrera AC, Martinez AC, Manes S. Dynamic redistribution of raft domains as an organizing platform for signaling during cell chemotaxis. *J Cell Biol* 2004;164:759–768. [PubMed: 14981096]
- Gomme PT, Bertolini J. Therapeutic potential of vitamin D-binding protein. *Trends Biotechnol* 2004;22:340–345. [PubMed: 15245906]
- Guo RF, Ward PA. Role of C5a in inflammatory responses. *Annu Rev Immunol* 2005;23:821–852. [PubMed: 15771587]
- Haddad JG, Hu YZ, Kowalski MA, Laramore C, Ray K, Robzyk P, Cooke NE. Identification of the sterol- and actin-binding domains of plasma vitamin D binding protein (Gc-globulin). *Biochemistry* 1992;31:7174–7181. [PubMed: 1643050]
- Head JF, Swamy N, Ray R. Crystal Structure of the Complex between Actin and Human Vitamin D-Binding Protein at 2.5 Å Resolution. *Biochemistry* 2002;41:9015–9020. [PubMed: 12119014]
- Huhtakangas JA, Olivera CJ, Bishop JE, Zanello LP, Norman AW. The vitamin D receptor is present in caveolae-enriched plasma membranes and binds 1 alpha,25(OH)2-vitamin D3 in vivo and in vitro. *Mol Endocrinol* 2004;18:2660–2671. [PubMed: 15272054]
- Kew RR, Fisher JA, Webster RO. Co-chemotactic effect of Gc-globulin (vitamin D binding protein) for C5a. Transient conversion into an active co-chemotaxin by neutrophils. *J Immunol* 1995a;155:5369–5374. [PubMed: 7594552]
- Kew RR, Mollison KW, Webster RO. Binding of Gc globulin (vitamin D binding protein) to C5a or C5a des Arg is not necessary for co-chemotactic activity. *J Leukoc Biol* 1995b;58:55–58. [PubMed: 7616106]
- Kew RR, Webster RO. Gc-globulin (vitamin D binding protein) enhances the neutrophil chemotactic activity of C5a and C5a des Arg. *J Clin Invest* 1988;82:364–369. [PubMed: 3392213]
- McVoy L. A., Kew R. R., 2005. CD44 and Annexin A2 Mediate the C5a Chemotactic Cofactor Function of the Vitamin D Binding Protein. *J. Immunol.* (in press).
- Merrifield CJ, Rescher U, Almers W, Proust J, Gerke V, Sechi AS, Moss SE. Annexin 2 has an essential role in actin-based macropinocytic rocketing. *Curr Biol* 2001;11:1136–1141. [PubMed: 11509239]
- Metcalf JP, Thompson AB, Gossman GL, Nelson KJ, Koyama S, Rennard SI, Robbins RA. Gc-globulin functions as a cochemotaxin in the lower respiratory tract. A potential mechanism for lung neutrophil recruitment in cigarette smokers. *Am Rev Respir Dis* 1991;143:844–849. [PubMed: 2008995]

- Mizwicki MT, Bishop JE, Olivera CJ, Huhtakangas J, Norman AW. Evidence that annexin II is not a putative membrane receptor for 1 α ,25(OH) $_2$ -vitamin D $_3$. *J Cell Biochem* 2004;91:852–863. [PubMed: 14991775]
- Mulkins MA, Manolagas SC, Deftos LJ, Sussman HH. 1,25-Dihydroxyvitamin D $_3$ increases bone alkaline phosphatase isoenzyme levels in human osteogenic sarcoma cells. *J Biol Chem* 1983;258:6219–6225. [PubMed: 6189822]
- Otterbein LR, Cosio C, Graceffa P, Dominguez R. Crystal structures of the vitamin D-binding protein and its complex with actin: Structural basis of the actin-scavenger system. *Proc Natl Acad Sci U S A* 2002;99:8003–8008. [PubMed: 12048248]
- Perez HD, Kelly E, Chenoweth D, Elfman F. Identification of the C5a des Arg cochemotaxin. Homology with vitamin D-binding protein (group-specific component globulin). *J Clin Invest* 1988;82:360–363. [PubMed: 3392212]
- Petrini M, Azzara A, Carulli G, Ambrogi F, Galbraith RM. 1,25-dihydroxycholecalciferol inhibits the cochemotactic activity of Gc (vitamin D binding protein). *J Endocrinol Invest* 1991;14:405–408. [PubMed: 1875017]
- Piquette CA, Robinson-Hill R, Webster RO. Human monocyte chemotaxis to complement-derived chemotaxins is enhanced by Gc-globulin. *J Leukoc Biol* 1994;55:349–354. [PubMed: 8120452]
- Robbins RA, Hamel FG. Chemotactic factor inactivator interaction with Gc-globulin (vitamin D-binding protein). A mechanism of modulating the chemotactic activity of C5a. *J Immunol* 1990;144:2371–2376. [PubMed: 2313096]
- Schwartz Z, Langston GG, Swain LD, Boyan BD. Inhibition of 1,25-(OH) $_2$ D $_3$ - and 24,25-(OH) $_2$ D $_3$ -dependent stimulation of alkaline phosphatase activity by A23187 suggests a role for calcium in the mechanism of vitamin D regulation of chondrocyte cultures. *J Bone Min Res* 1991;6:709–718.
- Senior RM, Griffin GL, Perez HD, Webster RO. Human C5a and C5a des Arg exhibit chemotactic activity for fibroblasts. *J Immunol* 1988;141:3570–3574. [PubMed: 3183381]
- Spudich JA, Watt S. The regulation of rabbit muscle contraction: biochemical studies of the interaction of the tropomyosin-troponin complex with actin and the proteolytic fragments of myosin. *J Biol Chem* 1971;246:4886–4871.
- Swamy N, Dutta A, Ray R. Roles Of the Structure and Orientation Of Ligands and Ligand Mimics Inside the Ligand-Binding Pocket Of the Vitamin D-Binding Protein. *Biochemistry* 1997;36:7432–7436. [PubMed: 9200691]
- Swamy N, Head JF, Weitz D, Ray R. Biochemical and preliminary crystallographic characterization of the vitamin D sterol- and actin-binding by human vitamin D-binding protein. *Arch Biochem Biophys* 2002;402:14–23. [PubMed: 12051678]
- Trujillo G, Kew RR. Platelet-derived thrombospondin-1 is necessary for the vitamin D binding protein (Gc-globulin) to function as a chemotactic cofactor for C5a. *J Immunol* 2004;173:4130–4136. [PubMed: 15356163]
- Verboven C, Rabijns A, De Maeyer M, Van Baelen H, Bouillon R, De Ranter C. A structural basis for the unique binding features of the human vitamin D-binding protein. *Nat Struct Biol* 2002;9:131–136. [PubMed: 11799400]
- White P, Cooke N. The multifunctional properties and characteristics of vitamin D-binding protein. *Trends Endocrinol Metab* 2000;11:320–327. [PubMed: 10996527]
- Zhang J, Kew RR. Identification of a region in the vitamin D-binding protein that mediates its C5a chemotactic cofactor function. *J Biol Chem* 2004;279:53282–53287. [PubMed: 15485893]
- Zigmond S, Hirsch J. Leukocyte locomotion and chemotaxis. New methods for evaluation and demonstration of a cell-derived chemotactic factor. *J Exp Med* 1973;137:387–410. [PubMed: 4568301]
- Zwahlen RD, Roth DR. Chemotactic competence of neutrophils from neonatal calves. Functional comparison with neutrophils from adult cattle. *Inflammation* 1990;14:109–123. [PubMed: 2323804]

Figure 1.

Effect of ligands on the binding of radioiodinated DBP to neutrophils and on the C5a co-chemotactic activity. **A.** Purified radioiodinated DBP (100 nM) was treated for 5 min at 37°C with and equimolar concentration of either G-actin, 25(OH)D₃, 1,25(OH)₂D₃, or G-actin plus 25(OH)D₃. Purified neutrophils (10⁷) were incubated in HBSS containing 0.1% BSA with the ¹²⁵I-DBP samples in a total volume of 0.1 ml. Samples were incubated at 37°C for 60 min after which cells were placed on ice and then washed twice with ice-cold assay buffer (1.0 ml each) and centrifuged for 7 min at 200 x *g* at 2°C. The cell pellets then were counted for total radioactivity in a gamma counter data are presented as fmols radioiodinated DBP bound either per 10⁶ neutrophils (*n* = 3–4). **B.** Purified DBP (100 nM) was treated for 5 min at 37°C with and equimolar concentration of either G-actin, 25(OH)D₃, 1,25(OH)₂D₃, or G-actin plus 25(OH)D₃. Neutrophils (4 x 10⁶/ml) were then preincubated for 40 min at 37°C with either DBP alone or DBP plus ligands in HBSS plus 1% BSA. Neutrophil movement then was assayed to 10 pM C5a or buffer for 30 min at 37°C. Numbers represent mean ± SEM of 3 experiments using cells from different donors. DBP plus C5a samples were significantly greater than the

corresponding C5a alone in all cases except as indicated by the asterisk where that sample is significantly ($p < 0.001$) less than all other C5a plus DBP samples.

Figure 2.

Dose response curves of DBP bound to 25(OH)D₃ or 1,25(OH)₂D₃. Purified DBP (100 nM) was pretreated with the indicated concentration of vitamin D for 5 min at 37°C. Neutrophils (4 × 10⁶/ml) were then preincubated for 40 min at 37°C with either DBP alone or DBP plus vitamin D in HBSS plus 1% BSA. Neutrophil movement then was assayed to 10 pM C5a or buffer for 30 min at 37°C. Numbers represent mean ± SEM of 3 experiments using cells from different donors. Neutrophils treated with DBP bound to 1,25(OH)₂D₃ (10⁻⁶ to 10⁻¹² M) migrated significantly less ($p < 0.001$) to C5a than corresponding samples containing 25(OH)D₃.

Figure 3.

Effect of bound versus free 1,25(OH)₂D₃ on C5a co-chemotaxis by DBP. Purified DBP (1 nM) was either treated for 5 min at 37°C with buffer (control), 100 pM 1,25(OH)₂D₃, 1 nM 25(OH)D₃ or 1 nM 25(OH)D₃ for 5 min followed by 100 pM 1,25(OH)₂D₃. Neutrophils (4 x 10⁶/ml) were then preincubated for 40 min at 37°C with either DBP alone or DBP plus vitamin D in HBSS plus 1% BSA. Neutrophil movement then was assayed to 10 pM C5a or buffer for 30 min at 37°C. Numbers represent mean ± SEM of 3 experiments using cells from different donors. Asterisk indicates that the sample is significantly ($p < 0.001$) less than all others.

Figure 4.

Effect of 1,25(OH)₂D₃ on chemotaxis to C5a, LTB₄, formyl peptide and CXCL8. Purified DBP (100 nM) was treated for 5 min at 37°C with 10 nM 1,25(OH)₂D₃. Neutrophils (4 × 10⁶/ml) were then preincubated for 40 min at 37°C with either DBP plus 1,25(OH)₂D₃ or buffer. Neutrophil movement then was assayed for 30 min at 37°C. Numbers represent mean ± SEM of 3 experiments using cells from different donors.

Figure 5.

Effect of sodium orthovanadate on the inhibitory action of 1,25(OH)₂D₃. Purified DBP (100 nM) was treated for 5 min at 37°C with 10 nM 1,25(OH)₂D₃. Neutrophils (4 x 10⁶/ml) were first pretreated for 15 min at 37°C with either 10 μM sodium orthovanadate (SOV) or buffer. Cells then were incubated for an additional 30 min at 37°C with either DBP plus 1,25(OH)₂D₃ or DBP alone (control). Neutrophil movement to 10 pM C5a was assayed for 30 min at 37°C. Numbers represent mean ± SEM of 3 experiments using cells from different donors. Asterisk indicates that the sample is significantly (p < 0.001) less than all others.