A receptor for the lipocalin placental protein 14 on human monocytes

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Abstract Human placental protein 14 (PP14), a member of the lipocalin structural superfamily, is an abundant amniotic fluid glycoprotein with documented immunoinhibitory activities. While receptors have been characterized for several other lipocalins, none have been reported to date for PP14. In the present study, two-color immunofluorescence and flow cytometry was used to screen peripheral blood mononuclear cell subpopulations for their capacity to engage fluoresceinated recombinant PP14. The tagged PP14 bound strongly in a specific and saturable fashion to $CD14^+$ (monocyte lineage) cells, but not to $CD20^+$ (B cell lineage) or $CD3^+$ (T cell lineage) cells. This binding was both pH- and temperature-sensitive, and was reduced by proteolytic pre-digestion of the cells with trypsin or proteinase K. Scatchard analysis demonstrated a single class of receptors on $CD14^+$ cells, with a K_D of $\sim 1\times10^{-8}$ and $\sim$10–35 000 receptors per cell. These findings constitute the first report of a cell surface-associated binding protein for PP14 and set the stage for exploring the molecular mechanisms of PP14-mediated signaling and immunomodulation.

Key words: Immunoregulation; Immunosuppression; Lipocalin

1. Introduction

Human placental protein 14 (PP14) is an abundant amniotic fluid protein produced by epithelial cells within the female and male reproductive tracts [1,2]. More recently, PP14 has been identified in hematopoietic cells, including normal megakaryocytes and platelets [3]. This 28-kDa glycoprotein, designated by assorted names in the literature (including progesterone-associated endometrial protein [4] and glycodelin [5]) is of considerable interest due to its remarkable immunomodulatory activities, with reported in vitro effects on inflammatory cytokine (IL-1 and IL-2) production [6,7] and T cell proliferation [3,8]. These immunoregulatory activities are evident at protein concentrations similar to those found in the serum of pregnant women [2]. This latter observation is especially significant, since it raises the intriguing possibility that PP14 may account, at least in part, for certain immunological phenomena associated with pregnancy (e.g. amelioration of autoimmune disease manifestations and skewing of cytokine profiles [9,10]).

Conserved sequence motifs and higher order structural features place PP14 into a family of proteins known as 'lipocalins.' The three-dimensional structure of lipocalins is typically characterized by an eight-stranded anti-parallel β-barrel (reminiscent of the calyx of a flower) that forms an internal pocket for binding small hydrophobic ligands [11]. Retinoids constitute potential ligands for several of the lipocalins, including retinol binding protein (RBP), β-lactoglobulin, α_1-microglobulin, and purpurin [11], as well as for PP14 (Mao, J. and Tykocinski, M., in preparation).

In addition to binding small hydrophobic ligands internally, lipocalins are also known to engage specific cell surface receptors. The receptor for RBP is best characterized. Bavik et al. cloned the cDNA of a 63-kDa RBP-binding membrane protein on bovine retinal pigment epithelial cells. [12–14]. Sivaprasadarao et al. showed specific binding of both native and recombinant RBP to human placental membrane vesicles [15] and have isolated 63- and 55-kDa receptor proteins using affinity chromatography [16]. This same group also identified a specific receptor for another lipocalin, odorant binding protein, on bovine nasal mucosa [17]. Receptors for α_1-microglobulin have been identified on both PMA-induced U937 cells [18] and mouse splenocytes [19]. Data also support the existence of a receptor for β-lactoglobulin (the closest lipocalin homologue to PP14) on calf intestine microvilli [20], as well as a receptor for purpurin [21].

Recently, we succeeded in producing human PP14 for the first time as a recombinant protein using a prokaryotic expression system (Mao, J. and Tykocinski, M., in preparation). Armed with this recombinant protein, we initiated an open-ended search for a receptor for PP14 on different hematopoietic cell types. We now report the identification of a cell surface binding protein for PP14 that is selectively expressed on the surface of $CD14^+$ cells of the monocytic lineage.

2. Materials and methods

2.1. Isolation of cells

Peripheral blood mononuclear cells (PBMC) were isolated from the blood of healthy volunteer donors by Ficoll-paque (Pharmacia Biotech, Piscataway, NJ, USA) density gradient centrifugation, as previously described [3]. Enrichment of a non-adherent monocyte population for use in ^{125}I-binding assays was accomplished by applying the total PBMC pool obtained in the initial Ficoll separation to a Percoll density gradient (Pharmacia) [22]. Cells were washed extensively prior to use. The resulting population consisted of 20–60% $CD14^+$ cells, as demonstrated by indirect immunofluorescence.

For experiments requiring enriched lymphoid subsets, PBMC were depleted of monocytes by adherence to plastic for 1 h at 37°C in RPMI-1640 (Gibco-BRL, Grand Island, NY, USA). The non-adherent cells were subsequently treated with LymphoKwik B (One Lamb-

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Abbreviations: IL-1, interleukin-1; PBMC, peripheral blood mononuclear cells; PHA, phytohemagglutinin; PP14, placental protein 14; RBP, retinol binding protein

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da, Canoga Park, CA, USA) for B cell enrichment or LymphoKwik T (One Lambda) for T cell enrichment, using protocols described by the manufacturer.

2.2. Generation of recombinant PP14

Recombinant human PP14 was generated using a prokaryotic expression system, to be described in detail elsewhere (Mao, J. and Tykocinski, M., in preparation). Briefly, BL2(DE3) *E. coli* were transformed with the expression construct pPP14.1·EE·his_6/ET-22b(+) (encoding full-length human PP14 cDNA with EE [23] and hexahistidine tags linked in tandem at the carboxyl terminus, and built upon the periplasmic expression vector pET-22b(+) (Novagen, Madison, WI, USA)). Fifty ml of an overnight culture grown in terrific broth (TB) [24] containing 100 μg/ml carbenicillin was used to inoculate 5 l of TB/carbenicillin. Cultures were incubated in a Bioflow 3000 biofermenter (New Brunswick Scientific, Edison, NJ, USA) at 37°C with stirring at 800 rpm and aeration until an A_{600} of 6–8 was reached. IPTG (5 g; Ambion, Austin, TX, USA) was then added to induce expression of recombinant PP14 protein. The cultures were grown at 37°C for an additional 3 h and then harvested by centrifugation. Cell pellets were resuspended in 2 l of 30 mM Tris-HCl (pH 8.0) and 20% sucrose and recovered by centrifugation. Pellets were subjected to osmotic shock by resuspending in 2 l of ice cold 5 mM $MgSO_4$, followed by 10 min of vigorous shaking. The mixture was centrifuged at $6000 \times g$ for 20 min at 4°C, and the recombinant hexahistidine-tagged PP14 was purified from the resulting supernatant in a single step by Ni^{2+}-Sepharose chromatography as described previously [25]. The protein was concentrated using a Centriprep 10 (Amicon, Beverly, MA, USA), and the purity of the protein was assessed by 12.5% reducing SDS-PAGE followed by Coomassie blue staining.

2.3. Proteins and antibodies

Other lipocalins used in binding assays included bovine milk β-lactoglobulin (Sigma, St. Louis, MO, USA), RBP purified from human urine (Sigma), α_1-microglobulin purified from human urine (Calbiochem, San Diego, CA, USA) and recombinant murine neutrophil gelatinase-associated lipocalin (NGAL; 24p3) [26]. Recombinant hexahistidine-tagged murine 24p3 was produced as a periplasmic protein by methods similar to those described above for PP14 (Weber, M. and Tykocinski, M., unpublished data). Proteins used as negative controls in competition assays included horse heart cytochrome *c* (Sigma) and bovine erythrocyte carbonic anhydrase (Sigma). In addition, recombinant soluble HLA-A2 α_3 domain, a hexahistidine-tagged, periplasmic protein [25] produced by methods similar to those used for recombinant PP14, was used as a negative control.

The mAbs used in binding assays included anti-leu-16 (an anti-CD20 mAb), anti-leu-M3 (an anti-CD14 mAb), mouse IgG1 and IgG2a (Becton-Dickinson). The hybridomas OKT3 (anti-CD3) and HP6001 (IgG2b isotype control) were obtained from the American Type Culture Collection (Rockville, MD, USA) and were used to raise ascites in pristane-primed mice. mAbs were purified from mouse ascites by affinity chromatography, as previously described [25].

2.4. Protein labeling

Recombinant PP14 (0.5–1 mg/ml) was incubated with 10 μg/ml fluorescein isothiocyanate (FITC) (Pierce Chemical, Rockford, IL, USA) in 100 mM carbonate, pH 9.0 for 1 h at RT. Unconjugated fluorescein was removed by buffer exchange into phosphate buffered saline using a Centricon 10 concentrator (Amicon). β-Lactoglobulin,

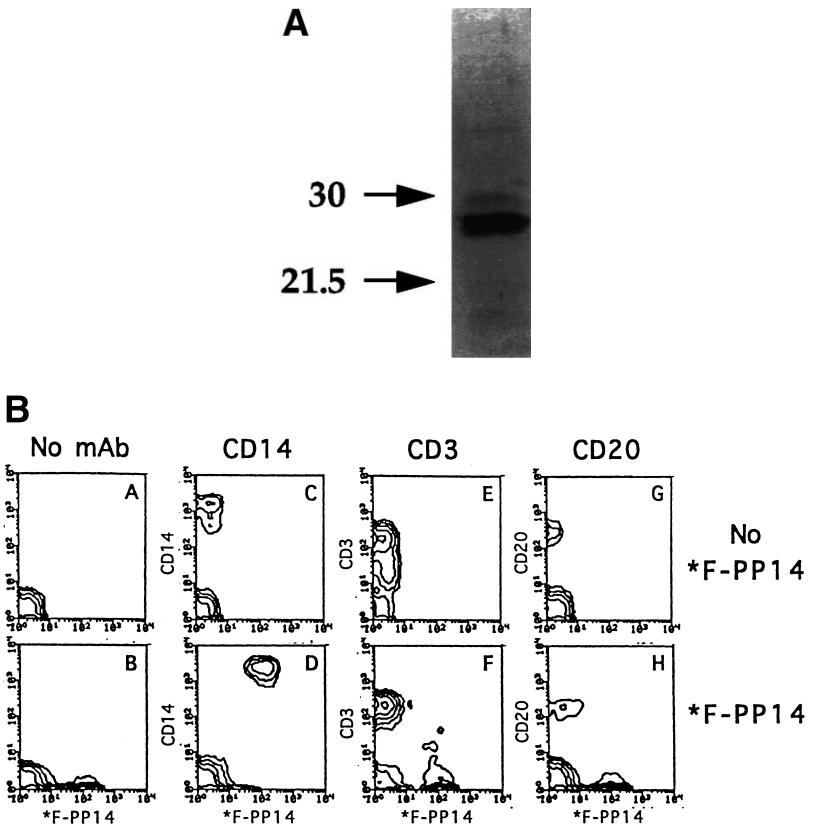

Fig. 1. A: SDS-PAGE analysis of purified recombinant PP14. Affinity-purified recombinant PP14 (see Section 2) was subjected to 12.5% SDS-PAGE under reducing conditions, followed by Coomassie Blue staining. Arrows corresponding to the 21.5- and 30-kDa size markers are shown at the left. B: *F-PP14 binds to the $CD14^+$ subpopulation of human PBMC. 10^6 PBMC were immunostained with mAb directed against surface markers for monocytes (anti-CD14, panels C and D), T cells (anti-CD3, panels E and F) or B cells (anti-CD20, panels G and H) plus a phycoerythrin-conjugated goat anti-mouse $F(ab')_2$, in the presence (panels B, D, F, H) or absence (A, C, E, G) of 1.5 μg/ml *F-PP14. Results are displayed as two-color flow cytometric contour plots with specific mAb staining on the *y*-axes and *F-PP14 binding on the *x*-axes. Only cells expressing CD14 bound *F-PP14 (panel D), whereas neither T (panel F) nor B (panel H) cells bound *F-PP14. Shown for comparison are unstained PBMC (panel A; secondary Ab only) and PBMC stained with *F-PP14 alone (panel B; along with secondary Ab). Additional isotype-matched control mAbs did not bind to PBMC (not shown). Shown are representative results from one of eight similar experiments.

horseheart cytochrome *c*, RBP, and recombinant murine 24p3 were fluoresceinated in a similar fashion.

Recombinant PP14 was ^{125}I labeled by the peroxidase method [27] using Na-^{125}I (NEN, Boston, MA, USA) and lactoperoxidase (Calbiochem). ^{125}I-labeled PP14 was separated from free ^{125}I by Sephadex G-25 (Pharmacia) gel filtration. Specific activities of $1.2–5.0\times10^6$ cpm/pmol were achieved. RBP and recombinant 24p3 were radio-iodinated in a similar fashion.

2.5. Binding assays

For fluorescent cytometric assays, 10^6 PBMC were incubated with fluoresceinated recombinant PP14 (*F-PP14) (final concentrations ranging from 50 to 150 nM) in RPMI-1640 supplemented with 0.75% bovine serum albumin and 0.05% sodium azide for 3 h at room temperature (RT). During the last 30 min of this incubation period, a 1:10 dilution of blocking pre-immune rabbit serum (Sigma) was added. The cells were then immunostained with one of several mAbs identifying specific mononuclear cell subpopulations for one hour on ice. Cells were washed with wash buffer (PBS with 0.5% BSA and 0.05% sodium azide), stained with phycoerythrin-conjugated goat anti-mouse secondary F(ab′)$_2$ (Boehringer-Mannheim, Indianapolis, IN, USA) for one hour on ice, and analyzed on a FACScan flow cytometer (Becton-Dickinson, San Jose, CA, USA). Specificity of binding was determined by competition assays in which 100- to 150-fold molar excess of recombinant PP14 or irrelevant control protein was added to the cells just prior to addition of the fluoresceinated ligand.

In some experiments, PBMC were pre-treated with either a solution containing trypsin 0.5 g/l and EDTA 0.2 g/l (Gibco-BRL), proteinase K 0.2 mg/ml (Bethesda Research Laboratories, Gaithersburg, MD, USA), 10 mM EDTA, or 2 mM dithiothreitol for 30 min at 37°C. After three washes, cells were stained with *F-PP14 as described above. In other experiments, PBMC were treated with 2 mu/ml endoglycosidase H (Genzyme Diagnostics, Cambridge, MA, USA) for 3 h at 37°C, followed by washing and staining with *F-PP14.

^{125}I-PP14 binding assays were performed as follows: 1.5 ml polypropylene microcentrifuge tubes (Laboratory Products Sales, Rochester, NY, USA) were pre-blocked with 2% BSA in PBS for 3 h at RT. 2×10^6 PBMC enriched for monocytes by Percoll density gradient centrifugation were incubated with ^{125}I-labeled PP14 in a total volume of 500 μl of binding medium (RPMI-1640 with 0.2% BSA and 0.1% NaN_3) for 3 h at RT in the presence of increasing concentrations of unlabeled recombinant PP14. Unlabeled PP14 was pre-incubated with the cells for 1 h at RT prior to the addition of labeled PP14. Cells were then pelleted by centrifugation to separate bound from unbound radioactive ligand. Pellets were washed twice with binding media, and bound radioligand was measured using a γ-counter (Beckman, Irvine, CA, USA). ^{125}I-24p3 and ^{125}I-RBP as ligand were used as negative controls. Competition studies were similarly performed using unlabeled α_1-microglobulin. Data was analyzed and Scatchard plots generated using the KELL for Windows program (Biosoft, Ferguson, MO, USA).

3. Results

Recombinant human PP14 was isolated from the periplasm of BL21(DE3) *E. coli* transformed with the bacterial expression vector pPP14.1·EE·his$_6$/ET-22b(+). The hexahistidine-tagged PP14 protein was affinity-purified from periplasmic extracts of these bacteria by metal chelate chromatography, and its relative purity was verified by Coomassie blue-stained SDS-PAGE (Fig. 1A). The non-glycosylated bacterial protein product migrated as a homogeneous band slightly faster than the estimated ∼28-kDa size of native *N*-glycosylated human PP14 [28].

Two-color immunofluorescence and flow cytometric analysis was used to screen different mononuclear cell subpopulations for their capacity to bind to fluorescein-conjugated PP14 (*F-PP14). The fluoresceinated PP14 ligand was used either alone or in combination with one of several mAbs directed against cell type-specific surface epitopes (CD3 as a pan-T cell marker, CD20 as a pan-B cell marker, and CD14 as a monocyte marker) or equal concentrations of isotype-matched control Abs. A representative experiment is shown in Fig. 1B.

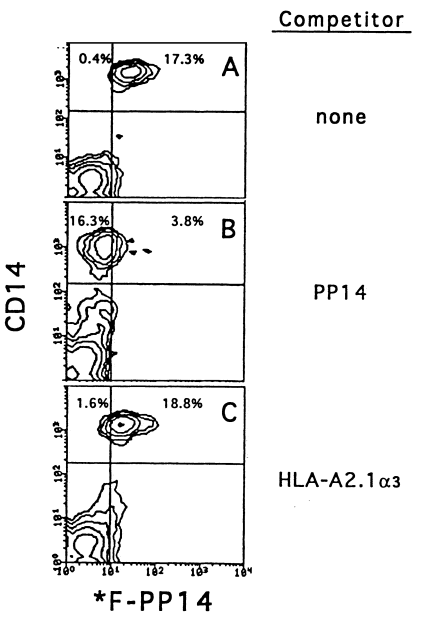

Fig. 2. Binding of *F-PP14 to CD14$^+$ monocytes is specific and saturable. 10^6 anti-CD14-stained PBMC were incubated with *F-PP14 (3 μg/ml) in the absence (panel A) or presence (panel B) of a 150-fold excess of unlabeled recombinant PP14. Unlabeled PP14 significantly inhibited binding of *F-PP14 to CD14$^+$ cells (panel B). Similar concentrations of an unlabeled control protein (HLA-A2.1α_3), failed to reverse binding of *F-PP14 to CD14$^+$ cells (panel C). Numbers shown correspond to percentage of cells analyzed that lie within the indicated sector.

When used alone, *F-PP14 bound to a proportion of unfractionated PBMC (Fig. 1B, panels A and B). In different donor samples, the percentage of ligand-binding cells ranged from 13 to 28%. Interestingly, *F-PP14 was found to selectively associate with the CD14$^+$ subset, corresponding to the monocytic cell fraction (Fig. 1B, panels C and D). The entire CD14$^+$ population shifted to the right in these flow cytometric contour plots, indicating homogeneous binding of the labeled ligand (Fig. 1B, panel D). In contrast, there was no significant binding of *F-PP14 to either CD3$^+$ T cells (Fig. 1B, panels E and F) or CD20$^+$ B cells (Fig. 1B, panels G and H).

Purified lymphocyte subpopulations were also investigated for binding to *F-PP14. Enriched T and B lymphocyte subpopulations depleted of monocytes by adherence and lysis by antibody and complement failed to bind *F-PP14 (data not shown). Isotype-matched control mAbs corresponding to the isotypes of the CD14, CD3 and CD20 mAbs did not bind to any of the cells.

The binding of *F-PP14 to CD14$^+$ monocytes was both specific and saturable. Binding was substantially reversed by adding a 150-fold excess of unlabeled recombinant PP14 (Fig. 2B). In contrast, similar concentrations of an irrelevant hexahistidine-tagged periplasmic protein (HLA-A2.1α_3) failed to reverse the binding of *F-PP14 to monocytes (Fig. 2C). CD14$^+$ monocytes also failed to bind *F-cytochrome *c* (not shown).

To test degeneracy of binding to the putative monocyte receptor among lipocalin family members, purified β-lactoglo-

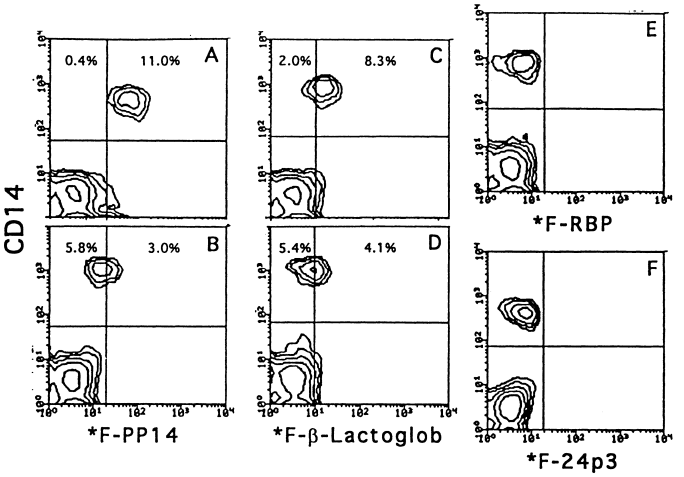

Fig. 3. Survey of lipocalin binding to CD14$^+$ monocytes. Two-color flow cytometric analysis was performed as described previously, detecting CD14 epitopes on the *y*-axes and fluoresceinated lipocalins on the *x*-axes. *F-β-lactoglobulin (1.7 μg/ml; panel C) bound to CD14$^+$ cells, though to a lesser extent than did *F-PP14 (2 μg/ml; panel A). A 100-fold excess of unlabeled PP14 inhibited binding of both *F-PP14 (panel B) and *F-β-lactoglobulin (panel D) to CD14$^+$ cells. Two other lipocalins, *F-RBP (2.5 μg/ml panel E) and *F-murine 24p3 (2 μg/ml; panel F), failed to bind to CD14$^+$ cells.

bulin, RBP, and murine 24p3 were each fluorescein-labeled and used as ligand probes. Fluoresceinated β-lactoglobulin bound to CD14$^+$ cells (Fig. 3C), though to a lesser degree than did *F-PP14 (Fig. 3A). The binding between *F-β-lactoglobulin and the CD14$^+$ monocytes was inhibited by a 100-fold excess of unlabeled recombinant PP14 (Fig. 3D). Conversely, the binding of *F-PP14 was also partially inhibited by an excess of unlabeled β-lactoglobulin (not shown). In contrast, neither *F-RBP (Fig. 3E) nor *F-24p3 (Fig. 3F) bound to the CD14$^+$ cells. Thus, lipocalins do not promiscuously bind to CD14$^+$ monocytes, although at least two lipocalins, PP14 and β-lactoglobulin, do so. Moreover, the cross-blocking findings suggest that the two bind to the same surface receptor. Similar cross-blocking has been observed for two other lipocalins (odorant-binding protein and major urinary protein) in binding to the receptor for odorant-binding protein [17].

The receptor on CD14$^+$ monocytes for *F-PP14 was further characterized. *F-PP14 binding to the cells was significantly reduced by protease digestion of PBMC with either trypsin (Fig. 4A) or proteinase K (Fig. 4B), substantiating that the receptor is a protein. While sensitive to proteolysis, the receptor's capacity to engage PP14 was not reduced by endoglycosidase H treatment of the PBMC. Preincubation of PBMC with EDTA or the reducing agent dithiothreitol failed to reduce the binding of *F-PP14 to monocytes (data not shown), suggesting that neither divalent cations nor disulfide bonds are essential for binding.

The temperature and pH sensitivity of *F-PP14 binding to monocytes were next evaluated. Significant binding was observed at both 37°C and 20°C, with less detectable binding at 0°C (Fig. 5A). Binding was also found to be pH-dependent (Fig. 5B). Maximal binding occurred at neutral pH (7.5), with decreased binding at lower and higher pHs. Taken together, the protease, temperature, and pH sensitivities are consistent with a polypeptide receptor for PP14.

In order to substantiate the findings with *F-PP14, ^{125}I-PP14 was used as ligand in radiometric assays. ^{125}I-PP14 bound to PBMC enriched for non-adherent monocytes, and cold competition with unlabeled PP14 demonstrated saturable

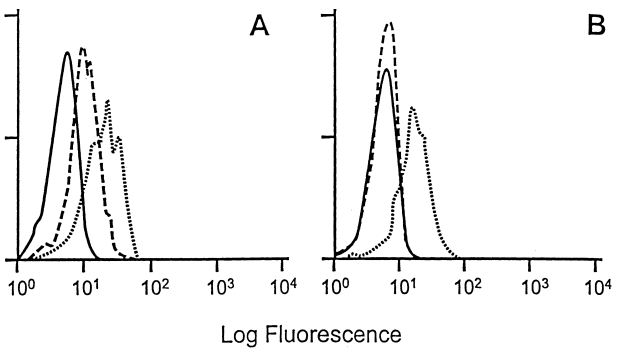

Fig. 4. Protease sensitivity of the PP14 receptor. 10^6 PBMC were pre-treated with either trypsin (panel A) or proteinase K (panel B), and subsequently stained with *F-PP14 (4.0 μg/ml). FACS histograms of monocyte-gated PBMC depicting *F-PP14 binding are shown. Legend: ———, unstained cells; - - -, protease-treated/*F-PP14-stained cells; · · ·, untreated/*F-PP14-stained cells.

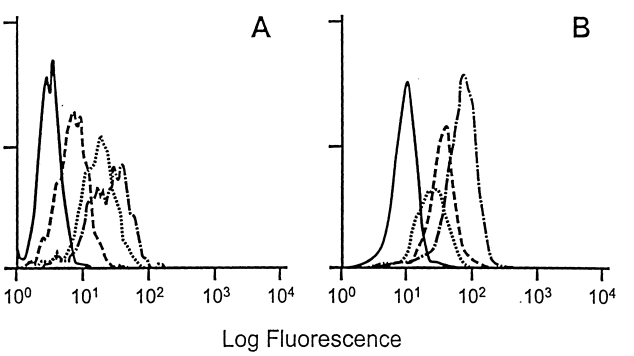

Fig. 5. Temperature dependency and pH sensitivity of PP14 binding to its receptor. Panel A: 10^6 PBMC were incubated with *F-PP14 (4 μg/ml) for 3 h at RT (· · ·), 37°C (- · - · -), or on ice (- - -), compared with unstained cells (———). FACS histograms depict staining of monocyte-gated PBMC with *F-PP14 at each temperature. Panel B: 10^6 PBMC were incubated with *F-PP14 (3 μg/ml) for 3 h at RT at pH 9 (· · ·), pH 6 (- - -), and pH 7.5 (- · - · -), compared with unstained cells (———). FACS histograms depict staining of monocyte-gated PBMC with *F-PP14 at each pH.

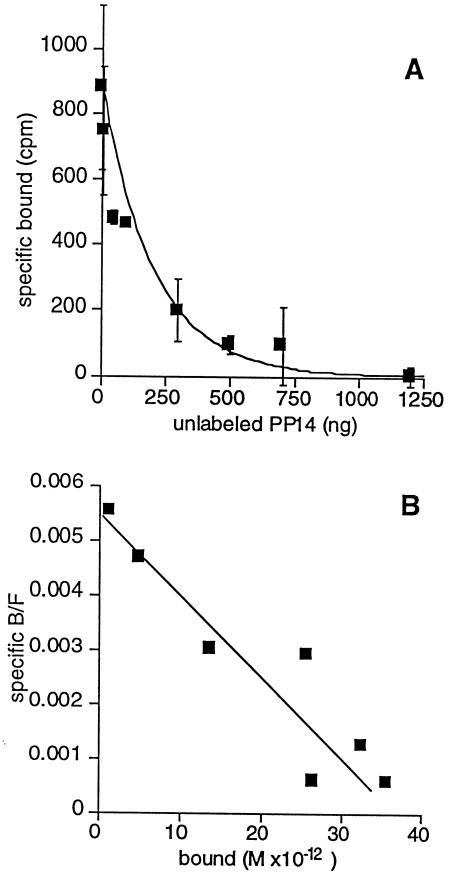

Fig. 6. Competition and Scatchard plots ^{125}I-PP14 binding to monocyte-enriched PBMC. A: ^{125}I-PP14 (6 ng/ml) was incubated with cells for 3 h at RT in the presence of increasing amounts of unlabeled recombinant PP14. After pelleting and washing of the cells, bound radioactivity within the pellets was determined. Non-specific binding (binding in the presence of >50 pmol unlabeled PP14) was 682 cpm. Each point represents the mean of duplicates ± S.E. Data is representative of results obtained in four similar experiments. B: The data from the competition curve in A was used to derive a Scatchard plot.

binding (Fig. 6A). Scatchard analysis for a representative experiment estimated a K_D of $0.67 \pm 0.11 \times 10^{-8}$ (Fig. 6B) with a range of $0.67–2.8 \times 10^{-8}$ in four similar experiments. Based on the assumption (supported by the flow cytometric data) that the recombinant PP14 is binding selectively to the CD14$^+$ subpopulation within the PBMC, the number of binding sites per cell ranged from 9510 to 35 820. ^{125}I-PP14 also failed to bind detectably to both purified T cells and the Jurkat T cell line (data not shown). Neither ^{125}I-RBP nor ^{125}I-24p3 bound significantly to PBMC (data not shown). Hence, the binding data with ^{125}I-PP14 and other iodinated lipocalins mirrored the cell binding selectivity of the fluoresceinated counterparts. Also of note, ^{125}I-PP14 binding to PBMC was not inhibited by cold competition with α_1-microglobulin, another lipocalin previously reported to bind to cells of monocytic lineage (data not shown) [18].

4. Discussion

The lipocalin PP14 is of considerable interest due to its abundance in amniotic and seminal fluids, presence in pregnancy serum and platelets, and intriguing immunomodulatory properties. In search of a receptor for this protein, we focused on PBMC, prompted by the immune connection. Recombinant human PP14, produced in a bacterial periplasmic expression system, was used here for the first time as a probe to screen for a PP14 surface receptor. The primary findings were as follows. (i) Using two-color flow cytometry, in conjunction with fluoresceinated PP14 as a probe, a receptor for PP14 was readily detected on all CD14$^+$ cells, an immunophenotype of the monocytic lineage; (ii) radiometric data using radio-iodinated PP14 as probe similarly pointed to the monocyte-enriched cell population as receptor-bearing cells; (iii) the radiometric analyses further established an approximate K_D of 1×10^{-8} for the interaction between recombinant PP14 and its monocytic receptor; (iv) the receptor for PP14 was shown to be protease- and pH-sensitive, consistent with its being a protein; and (v) a receptor for PP14 could not be detected on other cell types within PBMC when fluoresceinated periplasmic PP14 was used as a probe in this experimental system.

Receptors have been reported previously for several lipocalins, including RBP [12–16], β-lactoglobulin [20], purpurin [21], odorant binding protein [17], and α_1-microglobulin [18,19]. Interestingly, the receptor for α_1-microglobulin was detected on cells of the monocytic lineage [18]. However, α_1-microglobulin did not effectively compete for binding of ^{125}I-PP14 to monocyte-enriched PBMC, suggesting that these two lipocalins bind distinct receptors on monocytes. The present report adds PP14 and β-lactoglobulin to the list of lipocalins with receptors on monocytic cells. Of note, the previous report of a β-lactoglobulin receptor localized it to calf intestine microvilli [20]. Significantly, PP14 effectively competed with β-lactoglobulin for binding to the monocytic receptor. However, the precise relationship of the α_1-microglobulin, PP14, and β-lactoglobulin receptors remains to be determined.

A number of years ago, Bolton and coworkers reported that PP14 interferes with interleukin-1 (IL-1) production in phytohemagglutinin (PHA)-treated PBMC cultures [7]. Since monocytes are a primary source of IL-1, it is possible that PP14 negatively signals CD14$^+$ monocytes through the receptor identified in the present study, and thereby reduces IL-1 production by these cells. However, notwithstanding the simplicity of this model, other mechanisms cannot be ruled out at this stage. For example, the monocytic receptor could serve as a docking site for PP14, which upon PP14 engagement promotes the release of bioactive ligands (e.g. retinoids) that are transported within the PP14 hydrophobic pocket. With respect to the latter possibility, it is interesting to note that PP14 can selectively engage certain retinoids (Mao, J. and Tykocinski, M., in preparation) and that retinoids have documented immunosuppressive properties. Hence, 'docked' PP14 could serve as a local reservoir of an immunomodulatory ligand, or even as a reservoir for PP14 itself. This 'bioactive PP14 reservoir' model would parallel that proposed for the receptor for another immunosuppressive protein, the TGF-β type III receptor (betaglycan) [29]. Additional experiments will be required to sort through these functional possibilities.

The present study represents the first report of a PP14 receptor and sets the stage for its more detailed characterization at a molecular level. Insights into PP14's local and systemic activities should emerge.

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