The Human Erythrocyte Inflammatory Peptide (Chemokine) Receptor. Biochemical Characterization, Solubilization, and Development of a Binding Assay for the Soluble Receptor

Richard Horuk,*,‡ Timothy J. Colby,‡ Walter C. Darbonne,§ Thomas J. Schall,∥ and Kuldeep Neote∥

Departments of Protein Chemistry, Cardiovascular Biology, and Immunology, Genentech, Inc., 460 Point San Bruno Boulevard, South San Francisco, California 94080

Received November 18, 1992; Revised Manuscript Received January 11, 1993

ABSTRACT: In addition to the two human interleukin-8 (IL-8) receptors that have been cloned, IL-8RA and IL-8RB, we recently described a binding protein in human erythrocytes that binds IL-8 and monocyte chemotactic peptide-1 (MCP-1), which we have termed the chemokine (CK) receptor. This communication describes the biochemical characterization, detergent solubilization, and development of a solubilized receptor binding assay for the erythrocyte CK receptor. Competitive ^{125}I-IL-8 binding studies in cells transfected with IL-8RA and IL-8RB revealed that only IL-8 and MGSA were able to displace the radiolabeled IL-8 from these cells. In contrast, a whole array of chemokines were able to cross-compete with ^{125}I-IL-8 for binding to the CK receptor in erythrocyte ghosts. Scatchard analysis of ^{125}I-IL-8 binding to erythrocyte membranes and to dodecyl β-maltoside solubilized CK receptors revealed a single class of high affinity binding sites in both cases with K_D values of 9.5 nM ± 3.6 and 15.4 nM ± 5.0, respectively. Chemical cross-linking studies with erythrocyte membranes and with solubilized CK receptors indicated that the CK receptor has a lower molecular mass than the cloned IL-8 receptors (39 kDa compared to 57–69 kDa). Treatment of the cross-linked 47-kDA protein with N-glycanase reduced its molecular mass to 42 kDa.

The chemokines are a diverse group of secreted molecules that have a variety of biological properties including leukocyte chemotaxis and activation (Oppenheim et al., 1991; Yoshimura et al., 1987; Moser et al., 1990; Clark-Lewis et al., 1991; Moser et al., 1991). The family has been divided into two classes dependent on whether the first two conserved cysteine residues are separated by an intervening amino acid (C-X-C) or whether they are adjacent (C-C) (Schall, 1991). The C-X-C class members include interleukin-8 (IL-8),[1] MGSA, and platelet factor 4, while the C-C class includes RANTES, MCP-1, and the MIP-1 proteins (Moser et al., 1991). These chemokine superfamily members have been postulated to play a major role in both acute and chronic inflammation. Thus, knowledge of their biologic and physiological mechanisms of action together with the identification of specific cellular receptors will further our understanding of their mode of action in inflammatory diseases and help in designing strategies of pharmacologic intervention.

IL-8 is so far the most completely characterized of the chemokines, and a wealth of information is available regarding its biochemical properties (Oppenheim et al., 1991). Recently, two human IL-8 receptors termed the type A and type B receptors were identified from human neutrophil and HL-60 cDNA libraries (Holmes et al., 1991; Murphy et al., 1991). These two receptors, which show 77% amino acid identity, are members of the seven transmembrane family of receptors which are typically coupled to guanine nucleotide binding proteins. Both type A and type B receptors have been identified in human neutrophils (Lee et al., 1992). In addition, a high-affinity IL-8 receptor from rabbit was recently cloned that demonstrates specific binding for IL-8 but not to the related cytokine MGSA or to fMLP (Beckmann et al., 1991). In contrast to the cloned receptors described, we have characterized a promiscuous receptor on red blood cells that binds the C-X-C chemokines IL-8 and MGSA and the C-C chemokines MCP-1 and RANTES but not the C-C chemokines MIP-1α and MIP-1β (Darbonne et al., 1991; Neote et al., 1993). Due to the broad ligand specificity of the red blood cell receptor, we have designated it the multispecific chemokine (CK) receptor. Since the erythrocyte CK receptor has been shown to efficiently remove IL-8 from whole blood (Darbonne et al., 1991), it has been postulated that erythrocytes might play a major role in inflammation by limiting these, and other, chemokines in the circulation.

In the present communication, we compare and contrast the molecular properties of the erythrocyte CK receptor with the cloned IL-8 receptors. In addition, we describe here the detergent solubilization of the CK receptor and the design of a specific soluble receptor binding assay. Finally a variety of biochemical evidence is presented that suggests that the erythrocyte CK receptor is a unique receptor distinct from the IL-8 receptors that have been previously cloned.

EXPERIMENTAL PROCEDURES

Materials. Recombinant human IL-8 was expressed in *Escherichia coli* and purified as described previously (Hébert et al., 1990). ^{125}I-IL-8 (specific activity 143–180 μCi/μg) was labeled using lactoperoxidase as previously described (Hébert et al., 1990). Reagents for electrophoresis were purchased from Bio-Rad or Novex. N-Glycosidase F was obtained from Calbiochem.

* To whom correspondence should be addressed.
‡ Department of Protein Chemistry.
§ Department of Cardiovascular Biology.
∥ Department of Immunology.

[1] Abbreviations: IL-8, interleukin-8; RANTES, Regulated on Activation, Normal T Expressed and Secreted; CK, chemokine; IL-8RA, interleukin-8 receptor type A; IL-8RB, interleukin-8 receptor type B; RBC, red blood cells; DSS, disuccinimidyl suberate; HSAB, *N*-hydroxysuccinimidyl 4-azidobenzoate; EDC, 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride; PMSF, phenylmethanesulfonyl fluoride; PBS, phosphate-buffered saline; DTT, dithiothreitol; MGSA, melanocyte growth stimulating activity; MCP-1, monocyte chemotactic peptide-1; MIP-1, macrophage inflammatory protein.

0006-2960/93/0432-5733$04.00/0 © 1993 American Chemical Society

HEPES and all other reagent grade chemicals were from Sigma.

Isolation of Erythrocytes and Production of Erythrocyte Ghosts. Human erythrocytes were isolated from whole blood as described previously (Darbonne et al., 1991). Ghosts were prepared as described earlier (Steck et al., 1970).

^{125}I-Labeled IL-8 Binding to Erythrocyte Ghosts. Erythrocyte ghosts, 25–50 μg of membrane protein, were incubated with ^{125}I-labeled IL-8 (0.5 nM) and varying concentrations of unlabeled IL-8 at 37 °C for 1 h in PBS, pH 7.4. Binding was terminated by filtration through Whatman GF/C filters pretreated with 1% polyethyleneimine. Filters were rinsed three times with 2 mL of ice-cold PBS and counted in a γ counter (Iso-Data 100). Nonspecific binding was determined in the presence of 100 nM unlabeled ligand.

DNA Transfections. The expression vectors, pRK5.8 and pRK5.8rr.27-1.1, containing the IL-8RA and IL-8RB coding regions, respectively, were constructed as described (Lee et al., 1992). Human embryonic kidney 293 cells were transfected with the DNA for each of the two receptors by electroporation as described (Lee et al., 1992). The transfected cells were assayed after transient expression for 48 h.

Preparation of Soluble CK Receptors. Erythrocyte ghosts were suspended in 1% dodecyl β-maltoside, 25 mM Tris-HCl, pH 7.4 (containing 10 μg/mL each of leupeptin and aprotinin and 0.1 mM PMSF), to a final protein concentration of 5 mg/mL. The extract was incubated at 4 °C for 4 h in an end-over-end rotator, and insoluble material was removed by centrifugation at 100000*g* for 60 min. The clear supernatant was either used immediately or stored at −80 °C.

^{125}I-Labeled IL-8 Binding to the Solubilized CK Receptor. Specific ^{125}I-IL-8 binding to detergent-solubilized receptors was assayed in 25 mM Tris-HCl, pH 7.4 (containing 10 μg/mL each of leupeptin and aprotinin and 0.1 mM PMSF), 160 pM ^{125}I-IL-8, and a final detergent concentration of 0.12% dodecyl β-maltoside unless indicated otherwise in the text. After overnight incubation at 4 °C, bound ligand was recovered by rapid filtration through Schleicher and Schuell DEAE ion-exchange filters, followed by two rapid washes with 2 mL of ice-cold 25 mM Tris-HCl, pH 7.4. Radioactivity on the filters was determined by counting as above. Nonspecific binding was determined by the inclusion of 100 nM unlabeled IL-8 and was typically less than 2% of the total ^{125}I-IL-8 added.

Cross-Linking of ^{125}I-IL-8 to Erythrocyte Ghosts and to Soluble CK Receptor. Ghosts (150 μg) were incubated in the presence of 1 nM ^{125}I-IL-8, in the presence or absence of 100 nM unlabeled IL-8, for 1 h at 37 °C in PBS, pH 7.4. At the end of the incubation, the ghosts were pelleted by centrifugation (100000*g*, for 15 min), made up to the original volume in PBS, and chemically cross-linked with DSS, HSAB, or EDC, at a final concentration of 1 mM for 1 h at room temperature. The membrane suspension containing HSAB was placed on ice and irradiated for 2 min with a Rayonet RMR-400 photochemical reactor with the lamp placed 2 cm over the membrane suspension. The ghosts were then pelleted as described above and solubilized in SDS sample buffer in the presence of 50 mM DTT for 3 min at 20 °C and then analyzed by 10% SDS gels. To affinity label the soluble CK receptor, 120 μg of soluble membrane protein was incubated for 4 h at 4 °C in PBS, pH 7.4 (containing 10 μg/mL each of leupeptin and aprotinin and 0.1 mM PMSF), and 1 nM ^{125}I-IL-8 in the presence and absence of 100 nM unlabeled IL-8. The cross-linking agent EDC was added to a final concentration of 1 mM for 1 h at 4 °C. Aliquots of the cross-linked material were subjected to SDS–PAGE analysis as described above.

Treatment of ^{125}I-IL-8 Cross-Linked Erythrocyte Membranes with N-Glycosidase F. Erythrocyte membranes (150 μg) covalently labeled with ^{125}I-IL-8 were incubated with N-glycosidase F (20 milliunits) in 50 mM sodium phosphate buffer, pH 7, for 4 h at 37 °C. The membranes were centrifuged, solubilized, and analyzed by SDS–PAGE as described above.

Other Methods. SDS–PAGE was performed according to Laemmli (Laemmli, 1970) either on 1.5-mm slab gels or in Novex precast minigels. Resolving gels of 10% acrylamide were used. Prestained molecular weight marker proteins (Amersham) were used to estimate the molecular weight of the solubilized proteins. Gels were silver stained with a Bio-Rad silver stain kit according to the instructions of the manufacturer. Protein concentrations were determined using the Bio-Rad protein assay (Bradford, 1976). Binding data were analyzed by the Ligand program (Munson & Rodbard, 1980) as modified for the IBM PC (McPherson, 1983).

RESULTS

In order to examine the biochemical properties of the erythrocyte CK receptor, we prepared erythrocyte ghosts from human whole blood as described by Steck et al. (1970). Because of its availability, stability, and radiolabeling characteristics, ^{125}I-IL-8 was used to characterize the CK receptor in these studies. Initially we compared the ligand binding profile of the erythrocyte receptor to that of the cloned IL-8 receptors. Thus, we incubated cells transfected with the human type A and type B receptors (Lee et al., 1992) and erythrocyte ghosts with ^{125}I-IL-8 and determined whether we could displace the bound ligand with an excess of a variety of C-X-C and C-C unlabeled ligands (Figure 1A). Only IL-8 and MGSA were able to cross-compete for IL-8 binding to IL-8RA and IL-8RB. In contrast in erythrocyte ghosts, the C-C chemokine family members MCP-1 and RANTES were also able to cross-compete for IL-8 binding. However, MIP-1α, another C-C chemokine, failed to displace radiolabeled IL-8 from erythrocyte ghosts. These results confirmed the original observations of the promiscuous nature of the CK receptor using intact red blood cells (Neote et al., 1993) and, furthermore, showed that the CK receptor is different from the cloned IL-8 receptors.

In order to determine whether the CK receptor on erythrocyte ghosts and intact cells have similar biochemical properties, full dose–response curves of competitive ^{125}I-IL-8 binding to erythrocyte ghosts were carried out with each of the unlabeled ligands (Figure 1B). The competition curves were almost superimposable for all of the ligands, and only MIP-1α showed no displacement of ^{125}I-IL-8 (Figure 1B). As expected, Scatchard analysis of the competition curves yielded linear plots with almost identical K_D's for all of the unlabeled ligands; however, for maximum clarity only the displacement by unlabeled IL-8 is shown (Figure 1B, inset). The plot is linear, consistent with a single class of binding sites with a K_D of 9.5 nM ± 3.6 and a binding capacity of 2.6 ± 0.6 pmol of IL-8 binding sites/mg of membrane protein. These data are consistent with previously reported displacable binding of ^{125}I-IL-8, ^{125}I-MGSA, ^{125}I-MCP-1, and ^{125}I-RANTES and the lack of ^{125}I-MIP-1α binding in intact red blood cells (Darbonne et al., 1991; Neote et al., 1993). In addition, it shows that heterologous ligands apart from MIP-1α were able to cross-compete with each other, indicating binding to a common receptor (Neote et al., 1993).

The effect of guanine nucleotides on IL-8 binding to erythrocyte ghosts compared to that to plasma membranes

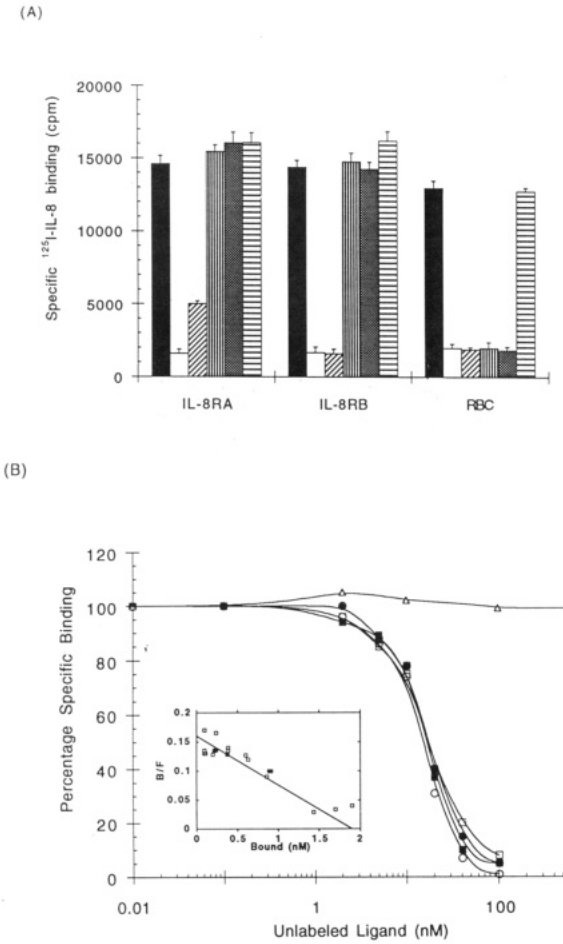

FIGURE 1: ^{125}I-IL-8 binding to erythrocyte ghosts. (A) Inhibition of ^{125}I-IL-8 binding to cells transfected with IL-8RA, IL-8RB, and erythrocyte ghosts. Cells were incubated for 1 h at 4 °C with ^{125}I-IL-8 in the absence of cross-competing ligand (solid boxes) or with 100 nM concentrations of unlabeled IL-8 (open boxes), MGSA (diagonal-striped boxes), MCP-1 (vertical-striped boxes), RANTES (cross-hatched boxes), and MIP-1α (horizontal-striped boxes). (B) Displacement of ^{125}I-IL-8 binding to erythrocyte ghosts. Ghosts were incubated for 1 h at 4 °C with ^{125}I-IL-8 in the presence of increasing concentrations of cross-competing ligands: IL-8 (●), MGSA (■), MCP-1 (○), RANTES (□), and MIP-1α (△).

prepared from cells transfected with the cloned IL-8 receptor was examined. Treatment of erythrocyte membranes with GTP up to a final concentration of 100 μM had no effect on ^{125}I-IL-8 binding: 10550 ± 991 compared to 10603 ± 683 cpm of IL-8 specifically bound in control compared to GTP-treated membranes. Treatment of erythrocyte membranes with 100 μM GTPγS, a nonhydrolyzable analog of GTP, also had no effect on IL-8 binding (data not shown). However, plasma membranes prepared from cells transfected with one of the cloned IL-8 receptors (Holmes et al., 1991) showed a 42% reduction in IL-8 binding when treated with 10 μM GTP: 11090 ± 1326 compared to 6400 ± 158 cpm of IL-8 specifically bound in control compared to GTP treated membranes, respectively. IL-8 binding in these membranes was also reduced by 10 μM GTPγS, but not by ATP (data not shown).

Erythrocyte membranes incubated with ^{125}I-IL-8, in the presence and absence of 100 nM unlabeled IL-8, were covalently labeled with three different cross-linking agents, HSAB, EDC, and DSS (Figure 2). All three agents covalently labeled a protein of approximate molecular mass 47 kDa, and the inclusion of unlabeled IL-8 decreased its intensity of labeling. Assuming that one IL-8 molecule binds to one

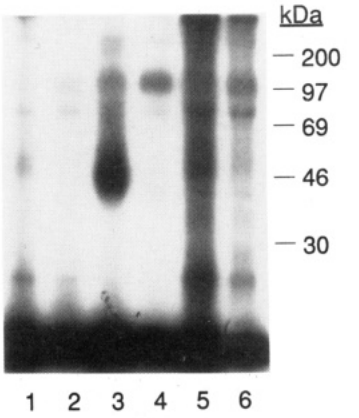

FIGURE 2: Ability of various cross-linking agents to covalently label erythrocyte CK receptors with ^{125}I-IL-8. Erythrocyte ghosts were incubated with 1 nM ^{125}I-IL-8 in the absence (lanes 1, 3, and 5) and in the presence (lanes 2, 4, and 6) of 100 nM unlabeled IL-8. After 1 h at 4 °C, the membranes were washed free of unbound label. The bound ^{125}I-IL-8 was cross-linked by incubation with HSAB (lanes 1 and 2), EDC (lanes 3 and 4), and DSS (lanes 5 and 6) for 1 h at 4 °C and then washed with PBS prior to analysis by SDS–PAGE. One hundred micrograms of protein was applied to the gel. After electrophoresis, the gels were dried down and subjected to autoradiography.

receptor molecule, the molecular mass of the CK receptor was estimated to be 39 kDa. The most efficient labeling of this band was achieved by the cross-linking agent EDC, and it was used in all further labeling experiments.

Erythrocyte ghosts were incubated with ^{125}I-IL-8 in the presence of increasing concentrations of unlabeled IL-8. When these membranes were covalently labeled by EDC, there was a dose-dependent reduction in the intensity of the 47-kDa protein band (Figure 3, top panel). The 47-kDa IL-8 cross-linked protein on the autoradiogram was scanned with a laser densitometer, and the total areas under the individual peaks were plotted as a function of IL-8 concentration (Figure 3, bottom panel). On the basis of this plot, the concentration of IL-8 to half-maximally displace ^{125}I-IL-8 from the erythrocyte CK receptor was 12 nM, which is in good agreement with the observed K_D for IL-8 binding (Figure 1).

The cross-linked CK receptor was incubated with N-glycanase for 4 h at 37 °C. The membranes were centrifuged, solubilized, and analyzed by SDS–PAGE as described above. As shown in Figure 4, treatment of the cross-linked CK receptor with N-glycanase reduced its molecular mass by 5 kDa, from 47 kDa to 42 kDa.

To define the optimal detergent conditions for the solubilization of the CK receptor in an active ligand-bindable form, we extracted erythrocyte membranes with a variety of detergents including Triton X-100, MEGA 8, CHAPS, Zwittergent, octyl β-glucoside, and dodecyl β-maltoside. Of these detergents, only dodecyl β-maltoside was successful in releasing the binding sites in an active form (data not shown). The most efficient extraction of CK receptor from erythrocyte membranes was achieved at a dodecyl β-maltoside concentration of 1.0%, which solubilized around 32% of the total membrane protein (Figure 5). In order to determine the optimal detergent concentration compatible with the IL-8 binding assay, we added increasing concentrations of dodecyl β-maltoside to membrane extracts solubilized with 1% detergent. The percentage of specific IL-8 binding to the detergent extracts was dependent on the final detergent concentration. Very little effect on IL-8 binding was observed by the addition of up to 0.4% dodecyl β-maltoside; however, at a concentration of 1%, the detergent decreased IL-8 specific binding by around 40% (data not shown).

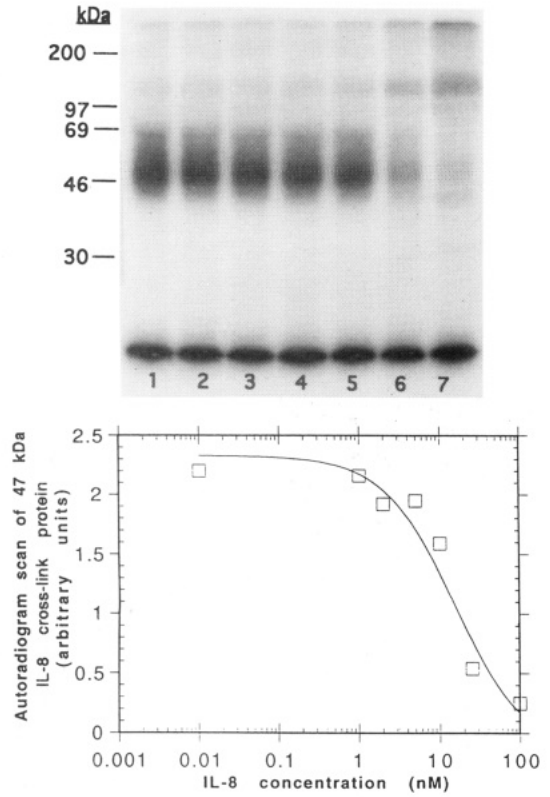

FIGURE 3: Effect of increasing concentrations of unlabeled IL-8 on the cross-linking of ^{125}I-IL-8 to erythrocyte CK receptors. Erythrocyte ghosts were incubated with 1 nM ^{125}I-IL-8 and the following concentrations of unlabeled IL-8: lane 1, none; lane 2, 1 nM; lane 3, 2.5 nM; lane 4, 5 nM; lane 5, 10 nM; lane 6, 25 nM; lane 7, 100 nM. After 1 h at 4 °C the membranes were washed free of unbound label. The bound ^{125}I-IL-8 was cross-linked by incubation with EDC for 1 h at 4 °C and then washed with PBS prior to analysis by SDS–PAGE. One hundred micrograms of protein was applied to each lane on the gel. After electrophoresis, the gel was dried and subjected to autoradiography. An autoradiogram from a typical experiment is shown in the top panel. The 47-kDa IL-8 cross-linked protein on the autoradiogram was scanned with a Molecular Dynamics laser densitometer. After integration, the total areas under the individual peaks were plotted as a function of IL-8 concentration and the resulting plot is shown in the bottom panel.

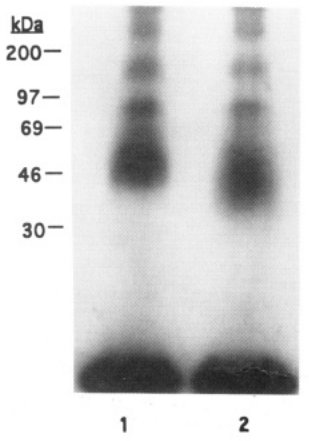

FIGURE 4: Effect of N-glycanase on the mobility of the ^{125}I-IL-8 cross-linked CK receptor on SDS gels. The erythrocyte CK receptor was cross-linked to ^{125}I-IL-8 as described above and treated with (lane 2) and without (lane 1) N-glycanase as described under Experimental Procedures. The reaction products were solubilized with SDS sample buffer and run on a 10% gel.

Scatchard analysis of ^{125}I-IL-8 displacement binding experiments with solubilized CK receptor (Figure 6) revealed that binding was linear, in agreement with binding to

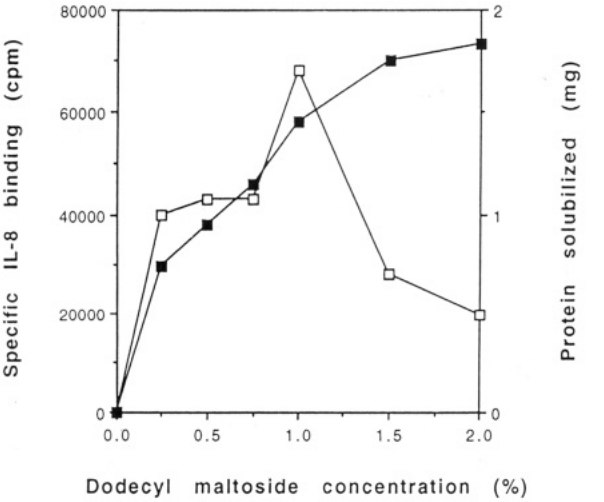

FIGURE 5: Solubilization of CK receptors from erythrocyte ghosts. Erythrocyte ghosts were extracted with increasing concentrations of dodecyl β-maltoside as described under Experimental Procedures. The ^{125}I-IL-8 binding activity of the extracts (□) was assayed at a final detergent concentration of 0.12%. Nonspecifically bound ^{125}I-IL-8 was less than 2% of the total ^{125}I-IL-8 added and has been subtracted. The protein concentration of the soluble extracts (■) was determined as described under Experimental Procedures.

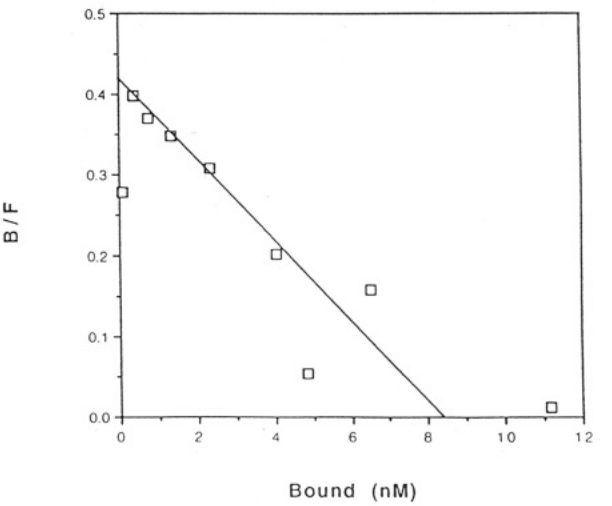

FIGURE 6: Scatchard analysis of ^{125}I-IL-8 binding to solubilized CK receptor. Solubilized receptor was incubated with ^{125}I-labeled IL-8 (0.25 nM) and varying concentrations of unlabeled IL-8. The Scatchard plot shown here is representative of three separate determinations. Binding shown represents specific binding. Nonspecific binding was around 2% of total ^{125}I-IL-8 added and has been subtracted.

erythrocyte membranes, with a K_D of 15.4 nM ± 5.0 and a binding capacity of 8.3 ± 1.1 pmol of IL-8 binding sites/mg of membrane protein. Comparison of the B_{max} values for the solubilized versus the membrane bound CK receptor, 8.3 compared to 2.6 pmol of binding sites/mg of protein, respectively, revealed a 3-fold enrichment of IL-8 binding sites per milligram of protein in solubilized receptors as compared with the membrane receptor. In addition, solubilization of erythrocyte membranes with dodecyl β-maltoside gives a high yield of receptor binding sites, with 70% of total IL-8 binding sites extracted. When competitive ^{125}I-IL-8 binding studies were carried out with solubilized CK receptors, the same pattern of displacement of the radiolabeled IL-8 was observed as in erythrocyte ghosts; i.e., 100 nM unlabeled MGSA, MCP-1, and RANTES were all able to fully cross-compete for IL-8 binding (Figure 7).

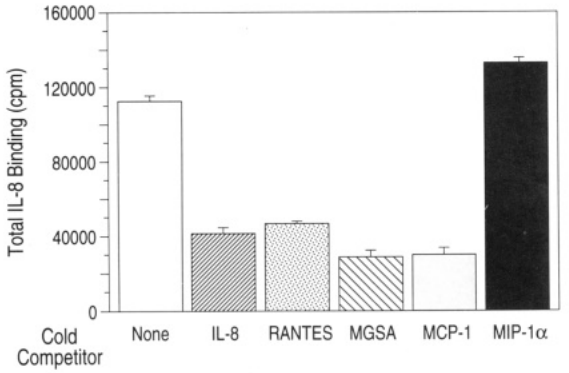

FIGURE 7: Inhibition of ^{125}I-IL-8 binding to solubilized CK receptor. Solubilized CK receptor was incubated for 12 h at 4 °C with ^{125}I-IL-8 in the absence of cross-competing ligand or with 100 nM concentrations of unlabeled IL-8, MGSA, MCP-1, RANTES, and MIP-1α. The vertical bar represents the SEM.

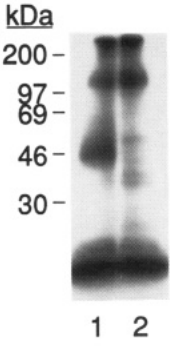

FIGURE 8: Cross-linking of ^{125}I-IL-8 to the solubilized CK receptor. Soluble CK receptor (120 μg) was incubated for 4 h at 4 °C in PBS, pH 7.4 (containing 10 μg/mL leupeptin and aprotinin and 0.1 mM PMSF), and 1 nM ^{125}I-IL-8 in the presence (lane 2) and absence (lane 1) of 100 nM unlabeled IL-8. The cross-linking agent EDC was added to a final concentration of 1 mM for 1 h at 4 °C. Aliquots of the cross-linked material were subjected to SDS–PAGE analysis.

The solubilized CK receptor was further characterized by covalent labeling with ^{125}I-IL-8 using the chemical cross-linking agent EDC. As shown in Figure 8, cross-linked solubilized CK receptor had a molecular mass of 47 kDa corresponding to that observed for the cross-linked membrane bound form of the CK receptor (Figure 2).

DISCUSSION

The present studies were initiated to biochemically characterize the newly identified, promiscuous human erythrocyte CK receptor. In this communication, we have demonstrated fundamental differences in the biochemical properties of the CK receptor compared to those of the cloned IL-8 receptors. Unlike specific chemokine receptors on monocytes or neutrophils, the erythrocyte CK receptor binds promiscuously to a wide array of both the C-C and C-X-C subgroups. We show by chemical cross-linking experiments that the CK receptor is a 39-kDa glycosylated protein. Preliminary experiments suggest that the CK receptor does not appear to be regulated by G-proteins. By contrast, the cloned IL-8 receptors have a much larger molecular mass and are coupled to G proteins (Holmes et al., 1991; Murphy et al., 1991). Furthermore, we have solubilized the CK receptor in an active form and demonstrated that the K_D for IL-8 binding and the cross-linking pattern for the detergent-solubilized receptor were similar to that of the membrane-bound receptor. The molecular properties of the CK receptor described above, together with its successful solubilization, should enable us to purify the protein in sufficient quantity to obtain amino acid sequence information.

The most efficient labeling of the CK receptor was achieved with EDC which activates carboxyl groups to nucleophilic attack by amino groups. The amino-reactive cross-linkers (HSAB and DSS) were less efficient in covalently attaching IL-8 to this receptor. Cross-linking of erythrocyte membranes incubated with ^{125}IL-8 in the presence of increasing concentrations of unlabeled IL-8 resulted in a dose-dependent reduction in the covalent labeling of the CK receptor (Figure 3, top panel). We found that the concentration range in which this occurred coincided with the range in which IL-8 interacts with the erythrocyte receptor (Figure 3, bottom panel) and induces chemotaxis in neutrophils (Clark-Lewis et al., 1991; Moser et al., 1991).

Using the solubilized binding assay described, we found that the erythrocyte CK receptor could be solubilized in a form that still retained maximal ability to bind ligand. Moreover, Scatchard (Scatchard, 1949) analysis of ^{125}I-IL-8 displacement binding experiments revealed that detergent solubilization had little effect on the binding affinity of the CK receptor since the K_D values for membrane and solubilized receptors were very similar and comparable with earlier reported values for intact erythrocytes (Darbonne et al., 1991).

A variety of cross-linking experiments in several cell types have demonstrated that IL-8 can be cross-linked to proteins of molecular mass 58–67 kDa (Moser et al., 1991; Samanta et al., 1989; Grob et al., 1990). Thus, the erythrocyte CK receptor, which has a calculated molecular mass of 39 kDa (Figure 2), is at least 19 kDa smaller. Furthermore, treatment of the cross-linked CK receptor with N-glycanase, which hydrolyzes the glycosylamine linkages between oligosaccharide chains and asparagine residues on a protein (Tarentino et al., 1985), changes its mobility on the gel from 39 kDa to 34 kDa. Given that the molecular mass of the cloned IL-8 receptors, based on the deduced protein sequence, would be 41 kDa (Holmes et al., 1991; Murphy et al., 1991), these data, as well as the ability of the CK receptor to bind to a variety of chemokines, support the idea that the erythrocyte CK receptor has a different structure compared to that of the cloned IL-8 receptors.

Guanine nucleotide binding proteins serve as transmembrane signal transducers for some chemoattractant receptors (Schepers et al., 1992), and the IL-8 receptors have been shown to be G-protein linked (Kupper et al., 1992). Our preliminary data with erythrocyte ghosts do not support the idea that the CK receptor is G-protein linked since IL-8 binding to erythrocyte ghosts showed no sensitivity to GTP or to GTPγS at concentrations which resulted in a 50% reduction in IL-8 binding to plasma membranes prepared from cells transfected with one of the cloned IL-8 receptors (Holmes et al., 1991). It is still possible, however, that the erythrocyte CK receptor retains the seven transmembrane domain so characteristic of this family of receptors but that it is uncoupled from its guanine nucleotide transducing unit. Alternatively, the erythrocyte CK receptor may have a unique three-dimensional protein structure compared to that of the cloned IL-8 receptors. Evidence in support of either of these two possibilities will have to await the purification and sequencing of this protein.

The physiological relevance of the promiscuous CK receptor on human erythrocytes is at present unclear. Given that it binds a whole array of chemokines, it might control the circulating levels of some chemokines and play an unsuspected role in regulating inflammation.

In summary, we have presented a variety of evidence that the erythrocyte CK receptor is a novel protein with molecular

properties that are significantly different from those of the known cloned IL-8 receptors. Furthermore, we have successfully solubilized the CK receptor in an active form with an affinity for IL-8 binding and molecular properties indistinguishable from that of its membrane-bound counterpart. These findings should enable us to design a protocol to purify this potentially novel protein and determine its primary amino acid sequence.

ACKNOWLEDGMENT

We thank Dr. Richard Vandlen for helpful suggestions and Gregg Bennett for the preparation of radiolabeled IL-8.

REFERENCES

Beckmann, M. P., Munger, W. E., Koslosky, C., VandenBos, T., Price, V., Lyman, S., Gerard, N. P., Gerard, C., & Cerretti, D. P. (1991) *Biochem. Biophys. Res. Commun. 179*, 784–789.

Bradford, M. (1976) *Anal. Biochem. 72*, 248–254.

Clark-Lewis, I., Moser, B., Walz, A., Baggiolini, M., Scott, G. J., & Aebersold, R. (1991) *Biochemistry 30*, 3128–3135.

Darbonne, W. C., Rice, G. C., Mohler, M. A., Apple, T., Hébert, C. A., Valente, A. J., & Baker, J. B. (1991) *J. Clin. Invest. 88*, 1362–1369.

Grob, P. M., David, E., Warren, T. C., DeLeon, P., Farina, P. R., & Homon, C. A. (1990) *J. Biol. Chem. 265*, 8311–8316.

Hébert, C. A., Luscinskas, F. W., Kiely, J. M., Luis, E. A., Darbonne, W. C., Bennett, G. L., Liu, C. C., Obin, M. S., Gimbrone, M. A., & Baker, J. B. (1990) *J. Immunol. 145*, 3033–3040.

Holmes, W. E., Lee, J., Kuang, W.-J, Rice, G., & Wood, W. I. (1991) *Science 253*, 1278–1280.

Kupper, R. W., Dewald, B., Jacobs, K. H., Baggiolini, M., & Gierschik, P. (1992) *Biochem. J. 282*, 429–434.

Laemmli, U. K. (1970) *Nature 227*, 680–685.

Lee, J., Horuk, R., Rice, G. C., Bennett, G., Camerato, T., & Wood, W. I. (1992) *J. Biol. Chem. 267*, 16283–16287.

McPherson, G. A. (1983) *Comput. Prog. Biomed. 17*, 107–114.

Moser, B., Clark-Lewis, I., Zwahlen, R., & Baggiolini, M. (1990) *J. Exp. Med. 171*, 1797–1802.

Moser, B., Schumacher, C., Tscharner, V., Clark-Lewis, I., & Baggiolini, M. (1991) *J. Biol. Chem. 266*, 10666–10671.

Munson, P. J., & Rodbard, D. (1980) *Anal. Biochem. 107*, 220–239.

Murphy, P. M., & Tiffany, H. L. (1991) *Science 253*, 1280–1283.

Neote, K., Darbonne, W., Ogez, J., Horuk, R., & Scholl, T. J. (1993) *J. Biol. Chem.* (in press).

Oppenheim, J. J., Zachariae, C. O. C., Mukaida, N., & Matsushima, K. (1991) *Annu. Rev. Immunol. 9*, 617–648.

Samanta, A. K., Oppenheim, J. J., & Matsushima, K. (1989) *J. Exp. Med. 169*, 1185–1189.

Scatchard, G. (1949) *Ann. N.Y. Acad. Sci. 52*, 660–672.

Schall, T. J. (1991) *Cytokine 3*, 165–183.

Schepers, T. M., Brier, M. E., & McLeish, K. R. (1992) *J. Biol. Chem. 267*, 159–165.

Steck, T. L., Weinstein, R. S., Strauss, J. H., & Wallach, D. F. H. (1970) *Science 168*, 255–257.

Tarentino, A. L., Gómez, C. M., & Plummer, T. H. (1985) *Biochemistry 24*, 4665–4671.

Yoshimura, T. K., Matsushima, K., Tanaka, S., Robinson, E. A., Apella, E., Oppenheim, J. J., & Leonard, E. J. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 9233–9237.