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Receptor Binding of Asialoerythropoietin

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Abstract The interaction of ^{125}I-asialoerythropoietin (asialoepo) with receptors has been characterized both by binding assay and affinity cross-linking. Purified spleen cells from mice infected with the anemia strain of Friend virus (FVA cells) have receptors for ^{125}I-asialoepo with two classes of affinity constant: one with Kd = 0.02–0.03 nM and 300–400 per cell, the other with lower affinity (Kd = 0.9–1.2 nM) and 1,000–1,200 per cell. The Kd value for the high affinity site is one-third of that for the binding of native ^{125}I-erythropoietin (^{125}I-epo) to the same FVA cells (Kd = 0.08–0.1 nM). Using ^{125}I-asialoepo or ^{125}I-epo affinity cross-linking methods, we find two components with apparent molecular weights of 88 kDa and 105 kDa in FVA cells, and in the transformed mouse cell lines, 201, IW32, and NN10, in agreement with earlier studies using ^{125}I-epo. These results indicate that ^{125}I-asialoepo binds to the same receptors as ^{125}I-epo, but with greater affinity for the high affinity site. Since 201 cells contain only a single class of lower affinity receptors for erythropoietin (epo), finding the same two components as found for FVA cells by cross-linking experiment indicates that the two components do not represent the two classes of receptor.

Key words: erythropoietin, receptor binding, affinity cross-linking

Erythropoietin (epo), a glycoprotein, stimulates the proliferation and differentiation of erythroid precursor cells after interacting with specific receptors. It has been demonstrated that with ^{125}I-epo as ligand there are two classes of epo receptors on purified spleen cells of mice treated with the anemia of Friend virus (FVA cells) (Sawyer et al., 1987) or their membrane preparations (Sawyer et al., 1987a), on erythroid progenitor cells from fetal mouse liver (Fukamachi et al., 1987), and on COS cells transformed with an epo receptor cDNA (D'Andrea et al., 1989), and that there is only one class of epo receptor (low affinity) on MEL cell lines (D'Andrea et al., 1989; Todokoro et al., 1987; Mayeux et al., 1987) and erythroid progenitors from fetal rat liver (Mayeux, et al., 1987a).

Asialoerythropoietin (asialoepo) has greater specific activity than native epo by in vitro assay, even though it has no activity in vivo (Goldwasser et al., 1974). This increased activity of asialoepo may be due to a higher affinity for receptors. Mufson and Gesner (1987) have shown that asialoepo is a more effective competitor for epo binding on erythroid precursors than is native epo. We have therefore investigated the properties of binding of native ^{125}I-epo and ^{125}I-asialoepo to FVA cells. We have also used ^{125}I-epo and ^{125}I-asialoepo affinity labeling method in FVA cells, PHZ cells, or transformed mouse cell lines (201, IW32, and NN10) to further characterize epo receptors.

METHODS

Chemicals and Reagents

The materials used are as follows: human recombinant epo (AMGen Inc., Thousand Oaks, CA); Na ^{125}I (Amersham, Arlington Heights, IL); disuccinimidyl suberate (DSS) and Iodo-Gen (1,3,4,6-tetrachloro-3α, 6α-diphenylglycoluril) (Pierce Chemical Co., Rockford, IL); phenylhydrazine hydrochloride (Eastman Organic Chemicals, Rochester, NY); Friend virus, the anemia strain SFFV$_A$/FRE cl-3/MULV (201) (gener-

Abbreviations used: Asialoepo, asialoerythropoietin; BSA, bovine serum albumin; DMEM, Dulbecco's modified essential medium; DMSO, dimethyl sulfoxide; DTT, dithiothreitol; DSS, disuccinimidyl suberate; epo, erythropoietin; FVA cells, purified spleen cells of mice infected with the anemia strain of Friend virus; Iodo-Gen, 1,3,4,6-tetrachloro-3α, 6α-diphenylglycoluril; kDa, kDaltons; PAGE, polyacrylamide gel electrophoresis; PHZ cells, purified spleen cells from mice injected with phenylhydrazine; SDS, sodium dodecyl sulfate.

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ously supplied by Dr. S.B. Krantz, Vanderbilt University); sodium dodecyl sulfate (SDS) (Bethesda Research Lab., Gaithersburg, MD); neuraminidase (type X-A)-agarose, dithiothreitol (DTT), and prestained SDS molecular weight standard mixture (Sigma Chemical Co., St Louis, MO); bovine serum albumin (BSA) (Calbiochem, San Diego, CA); and dimethyl sulfoxide (DMSO) (Fisher Scientific, Fair Lawn, NJ).

Cells

FVA cells were prepared from the spleens of BALB/C mice infected with 10^4 spleen focus-forming units of the anemia strain of the Friend virus by tail vein injection as described earlier (Koury et al., 1984). Spleens were harvested 14–21 days after the injection, and a single cell suspension made by gently pressing spleens against a wire mesh (60 gauge) in Iscove's modified Dulbecco's medium (IMDM) with 20 mM HEPES (pH 7.3) and 2% BSA. The cells were layered over 10% BSA in a phosphate buffer solution (NaCl 130 mM, KCl 5 mM, Na_2HPO_4 5 mM, KH_2PO_4 4 mM, pH 7.2) and collected by sedimentation at 50*g* for 5 minutes at 4°C. PHZ cells were obtained from spleens of BDF1 mice injected intraperitoneally with phenylhydrazine hydrochloride (60 mg/kg body weight) daily for two consecutive days. Spleens were harvested 72 hours after the second PHZ injection, and cells were separated as for FVA cells. The cloned erythroleukemic cell lines IW32 and NN10 were originally isolated from mouse spleens infected with a Friend murine leukemia virus (FMuLV) stock (I^{-5} virus stock) (Tambourin et al., 1983; Choppin et al., 1985). The erythroleukemic cell line 201 was induced by a biologically cloned FMuLV (clone 201) in E. Scolnick's laboratory from a complete Friend virus (anemic strain) (Choppin et al., 1985). All three cell lines were obtained from Dr. B. Varet at Hopital Cochin, Paris.

Radioiodination

Radioiodination was performed according to Fracker and Speck (1978). In brief, 0.5 μg of epo and 200 μCi of Na ^{125}I were added to a microsample vial (American Scientific Products, McGaw Park, IL) coated with 10 μg of Iodo-Gen. The mixture was left at 22°C for 30 seconds, then transferred to a tube containing 200 μl of KI (10 mg/ml in 0.5 M phosphate buffer pH 7.0) and 300 μl of column buffer (0.1 M phosphate, 0.1 M NaCl, 0.05% gelatin) with 3 mM sodium thiosulfate, ^{125}I-epo was separated from free $^{125}I^-$ on a G-25 column. The specific activity of the iodinated epo was 50 μCi/μg, equivalent to 2 atoms of iodine per epo molecule.

Preparation of ^{125}I-Asialoepo

Neuraminidase bound to agarose beads (10–20 units per gram of agarose) was washed twice with distilled water, then with 0.5 M phosphate buffer (pH 7.0) containing 0.01% gelatin. Four picomoles of ^{125}I-epo were incubated with 0.25 units of the washed neuraminidase-agarose in the phosphate buffer at 37°C for 1 hour with constant agitation. The agarose-bound enzyme was removed by centrifugation. The completeness of desialation was determined by gel electrophoresis, followed by autoradiography. The apparent molecular weights are 37 kDa for epo and 30 kDa for asialoepo. The molecular weight of native epo is 30.4 kDa (Davis et al., 1987) and that of asialoepo is calculated to be 27.4 kDa. The difference between their apparent and calculated molecular weights is probably due to the anomalous migration of these glycoproteins, especially epo, which contains sialic acid residues. There is no evidence of proteolytic enzyme contamination in the neuraminidase we used, since analysis by SDS gel electrophoresis yields a discrete band at M_r 30 kDa (Fig. 1).

Binding Assay

Equilibrium binding of the ligands to FVA cells or 201 cells was performed in duplicate or triplicate, in polypropylene tubes (5.0 ml) containing 0.5–3 × 10^6 cells, 0.004–3.0 nM ^{125}I-asialoepo or 0.01–3.0 nM ^{125}I-epo, with or without 300 nM unlabeled epo. The incubation medium was IMDM lacking sodium bicarbonate but containing 20 mM HEPES (pH 7.3) and 0.5% BSA, and the total volume was 100 μl. Samples were incubated for 10 hours at 4°C with shaking. The whole sample was transferred to a 1.5 ml Eppendorf tube containing 0.5 ml of dibutyl phthalate, centrifuged for 1 minute at 15,000*g*, and immediately frozen in liquid nitrogen. The tip with the cell pellet was cut off and radioactivity was determined in a gamma counter. Specific binding was defined as the amount of labeled epo or asialoepo displaceable by 300 nM unlabeled epo.

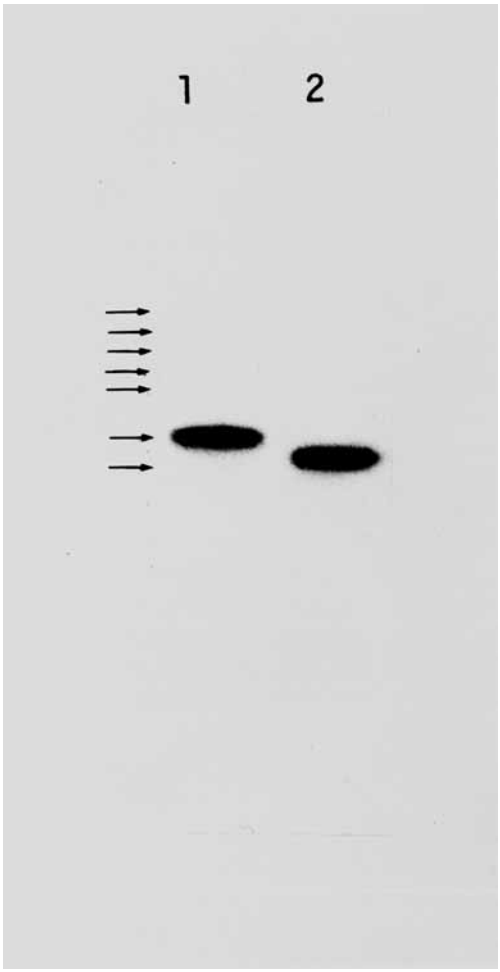

Fig. 1. Autoradiogram of ^{125}I-epo and ^{125}I-asialoepo. ^{125}I-epo (**lane 1**) and ^{125}I-asialoepo (**lane 2**) were subjected to 15% SDS-PAGE under reducing conditions, followed by autoradiography. Arrows from top to bottom indicate standard molecular markers, 180, 116, 84, 58, 48.5, 36.5, and 26.6 kDa. The apparent molecular weights of ^{125}I-epo and ^{125}I-asialoepo are 37 and 30 kDa, respectively.

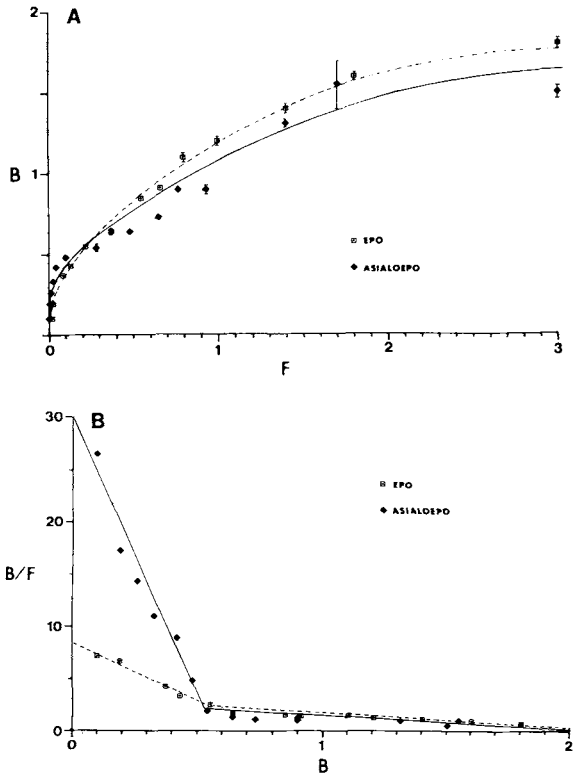

Fig. 2. (**A**) Specific ^{125}I-asialoepo binding to FVA cells. FVA cells (0.5×10^6 in 100 μl) were incubated at 4°C for 10 hours with various concentrations of ^{125}I-epo or ^{125}I-asialoepo. Specific binding was defined as the ^{125}I-epo or ^{125}I-asialoepo binding displaceable by 300 nM unlabeled epo. Vertical bars indicate standard errors, n = 3. At points where bars are not shown, the standard errors are within the symbol. Non-specific binding for epo ranged from 5% of total at 0.014 nM to 46% of total at 3.0 nM. Non-specific binding for asialoepo ranged from 2% of total at 0.0037 nM to 45% of total at 3.0 nM. Abscissa, concentration (nM); ordinate, bound (fmoles/10^6 cells); (**B**) Scatchard analysis of the same data. Abscissa: bound (fmol/10^6 cells); ordinate: bound/free (fmol/10^6 cells/nM). The standard errors are less than 10% of the means, n = 3.

Affinity Cross-Linking

Cells (10^6–10^7) were incubated with 0.5 nM ^{125}I-epo or ^{125}I-asialoepo in 150 μl of IMDM with 20 mM HEPES or Hank's balanced salt solution (HBSS) (pH 7.5) containing 0.2% BSA at 22°C for 2 hours with agitation, then washed twice with cold HBSS (pH 7.5). Cells were resuspended in 1 ml of HBSS (pH 7.8), and placed at 22°C. Freshly prepared DSS solution (0.1 M in DMSO) was added at a 1:100 dilution. After 18–20 minutes, the cross-linking reaction was stopped by the addition of 20 μl of 1.0 M glycine. The cross-linked cells were washed twice in HBSS (pH 3.0) and lysed using a solution containing 8.0 M urea, 2% Nonidet P-40, and 80 mM DTT. Twenty microliters of the lysing solution per million cells were used. The samples were left on ice for 2 minutes and centrifuged at 15,000*g* for 1 minute to remove debris and stored at −80°C. After thawing, an equal volume of 2 × loading buffer (120 mM Tris-HCl pH 6.8, 20% glycerol, 4% SDS, 0.02% bromphenol blue, with or without 80 mM DTT) was added. The solution was boiled for 2 minutes. The samples, along with prestained molecular weight markers, were subjected to electrophoresis according to Laemmli (1970). The stacking gel was 3.75% acrylamide (37.5:1.0) and the separating gel 7.5% acrylamide (37.5:0.5). Gels were dried and subjected to autoradiography on Kodak XAR-5 film

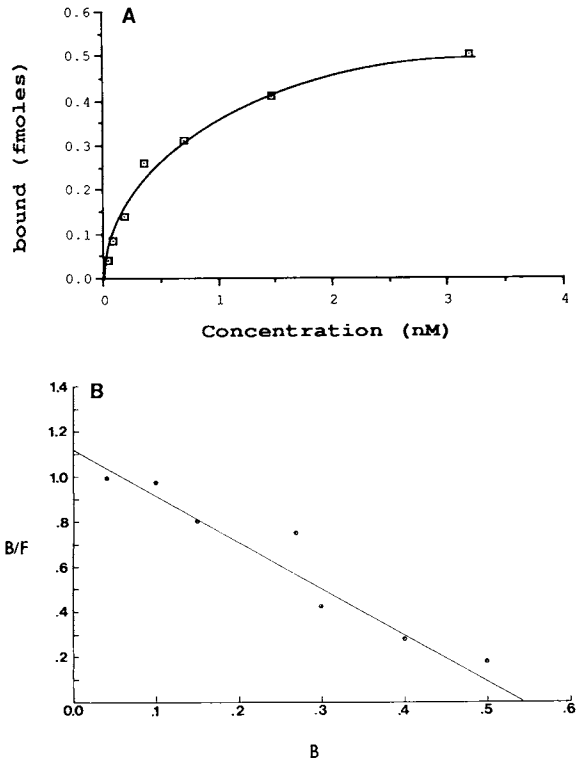

Fig. 3. (A) Specific ^{125}I-epo binding to 201 cells. Cells (3×10^6 in 100 μl) were incubated at 4°C for 10 hours with various concentrations of ^{125}I-epo. Specific binding was defined as the ^{125}I-epo binding displaceable by 300 nM unlabeled epo. The standard errors are less than 10% of the means, n = 3. Nonspecific binding ranged from 9% of total at 0.043 nM to 42% of total at 3.2 nM; (B) Scatchard analysis of the same data. Abscissa: bound (fmol/10^6 cells); ordinate: bound/free (fmol/10^6 cells/nM). The standard errors are less than 10% of the means, n = 3.

at −80°C with an intensifying screen. The exposure time was 3–7 days.

Densitometry

Densitometric analysis was performed using a 2202 Ultroscan Laser Densitometer (Pharmacia LKB Biotechnology Inc., Piscataway, NJ).

RESULTS

Binding of Radioiodinated epo and Asailo epo to FVA Cells

Both ligands were bound to FVA cells in a saturable manner, the binding data with both forms of epo could be expressed as two component Scatchard plots. Typical plots are shown in Figure 2a and 2b. In three separate sets of experiments, we have found with native epo as ligand, the higher affinity receptor has an apparent Kd of 0.08–0.1 nM and about 300–400 per cell, while the lower affinity class has an apparent Kd of 0.8–1.3 nM and about 900–1,200 per cell. For asialoepo as ligand the values are about 300–400 receptors at Kd = 0.02–0.03 nM and about 1,000–1,200 at Kd = 0.9–1.2 nM.

Binding of ^{125}I-epo to 201 Cells

The cell line 201, though unresponsive to epo, possesses specific epo binding sites (Fig. 3a). Scatchard analysis reveals that these cells have a single class of epo receptors with an apparent Kd of 0.55 nM and 360 per cell (Fig. 3b).

Affinity Cross-Linking Experiments

In order to further characterize epo receptors using asialoepo, we did affinity cross-linking experiments, followed by electrophoresis and autoradiography. Figure 4a and 4b shows that under reducing conditions there are two major components labeled with iodinated asialoepo, both in FVA and 201 cells. They have approximate molecular weights of 135 and 118 kDa. After subtracting 30 kDa of asialoepo, these two major components have apparent molecular weights of 105 and 88 kDa. The two bands were also detected in IW32 and NN10 cells. They were less dense than those in 201. This is probably due to the fact that IW32 and NN10 produce and secrete epo (Choppin et al., 1985), which will compete with radioactive asialoepo. In PHZ cells, however, we found a component of 95 kDa in place of the 88 kDa. The two bands in 201, IW32, and NN10 cells show equal intensity by densitometry, whereas in FVA and PHZ cells the ratio of intensity of the 88 kDa component to that of the 95 kDa component is 1.5. With ^{125}I-epo as the ligand, two bands (142 and 125 kDa) were detected in FVA and 201 cells (Fig. 5a and 5b). In FVA cells, the 125 kDa band is more intense than the 142 kDa band, and densitometry also shows a ratio of about 1.5 for the 125 and 142 kDa bands. Under the conditions of electrophoresis used, the apparent molecular

Fig. 4. (A) Autoradiogram of ^{125}I-asialoepo cross-linked receptors in FVA and PHZ cells. Cells were incubated with 0.5 nM ^{125}I-asialoepo in the presence or absence of excess unlabeled epo at 22°C for 2 hours. Cells were then cross-linked with DSS and solubilized. The samples were subjected to SDS-PAGE under reducing conditions, followed by autoradiography. Arrows from top to bottom indicate 135, 125, and 118 kDa bands; (B) Autoradiogram of ^{125}I-asialoepo cross-linked epo receptors in 201, IW32, and NN10 cells. Arrows from top to bottom indicate 135 and 118 kDa bands.

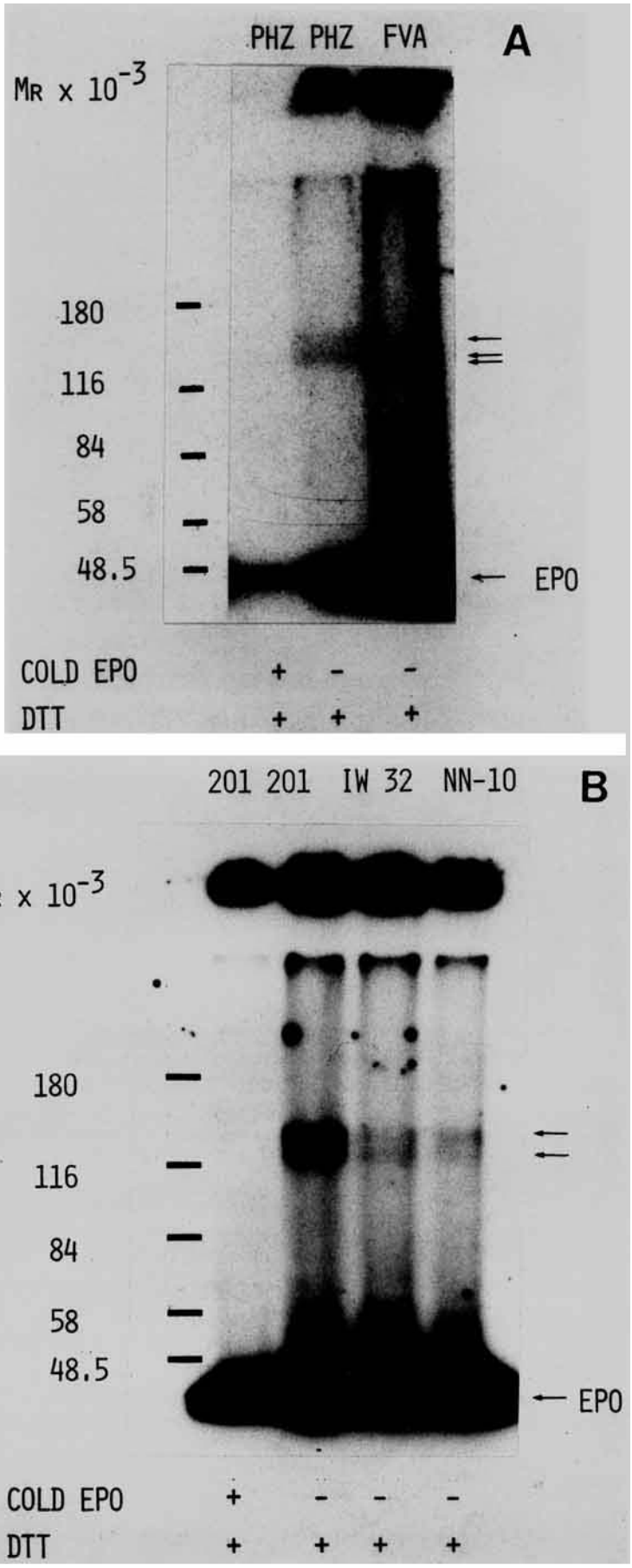
PHZ PHZ FVA
A
MR x 10-3
180
116
84
58
48.5
EPO
COLD EPO + - -
DTT + + +
201 201 IW 32 NN-10
B
MR x 10-3
180
116
84
58
48.5
EPO
COLD EPO + - - -
DTT + + + +

Figure 4.

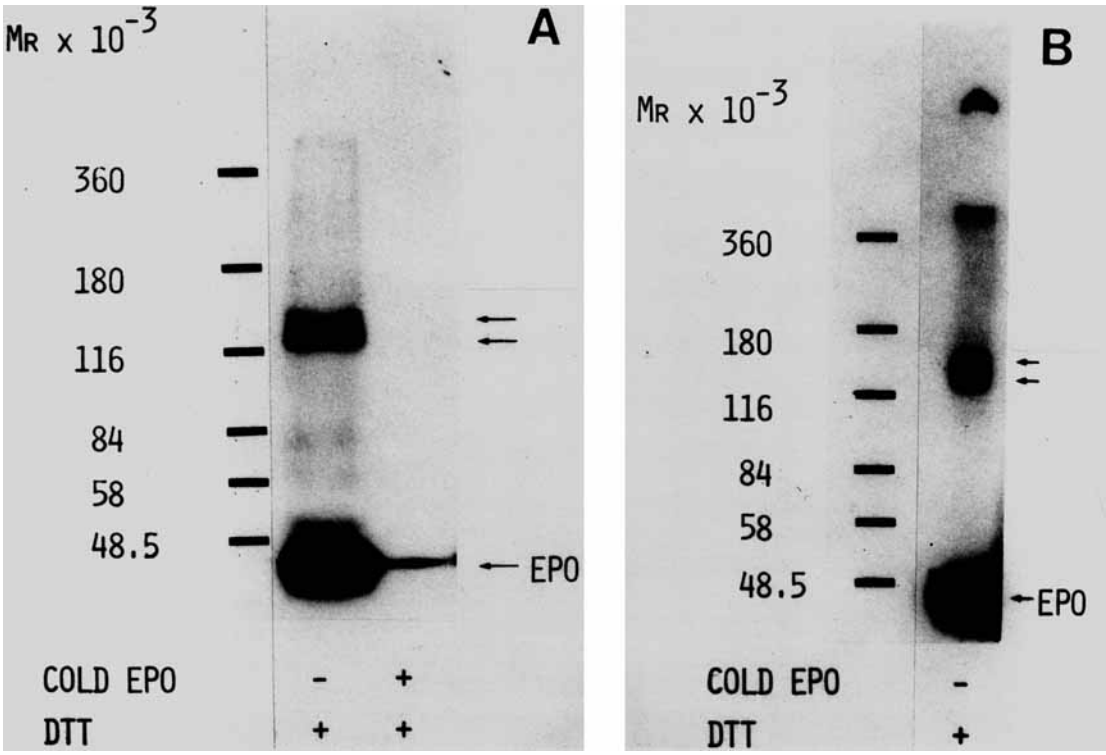

Fig. 5. (A) Autoradiogram of ^{125}I-epo cross-linked epo receptors in FVA cells. FVA cells were incubated with 0.5 nM ^{125}I-epo in the presence or absence of excess unlabeled epo at 22°C for 2 hours. Cross-linking with DSS and solubilization were performed. The samples were subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions, followed by autoradiography. Arrows from top to bottom indicate 142 and 125 kDa bands; (B) Autoradiogram of ^{125}I-epo cross-linked epo receptors in 201 cells. 201 cells were incubated with 0.5 nM ^{125}I-epo in HBSS containing 0.2% BSA at 22°C for 2 hours. Cells were then cross-linked with DSS and solubilized in lysis solution. The samples were subjected to SDS-PAGE under reducing conditions, followed by autoradiography. Arrows from top to bottom indicate 142 and 125 kDa bands.

weight of ^{125}I-epo is 37 kDa. After subtracting that value, the two major components show apparent molecular weights of 105 and 88 kDa. Excess unlabeled epo competed for the labeling of both bands (Fig. 5a), which indicates specific cross-linking. Some minor radioactive bands were also detected in the gel. Neither of the specific receptor components was detected in mouse thymus cells (Fig. 6), which show no specific epo binding.

DISCUSSION

In a recent paper, Imai et al. (1990) report that PHZ cells bind asialoepo at a much greater rate than native epo, with however essentially the same k_D values (250 and 290 pM, respectively). Since they did not report finding a high affinity receptor, we cannot compare their results with those reported here. Sawyer et al. (1987) found two populations of epo receptors in FVA cells using iodinated epo. The higher affinity site has a Kd of 0.09 nM and about 300 receptor molecules per cell; the lower affinity site has a Kd of 0.57 nM and about 650 molecules per cell. Our results are consistent with those findings. Sasaki et al. (1987), however, have detected only one class of binding site on FVA cells with a Kd of 0.24 nM. As they indicate, they have not done binding studies in the range of ligand concentration where a significant number of the lower affinity sites would interact with the ligand.

These experiments show that asialoepo binds to epo receptors in FVA cells in a manner similar to epo: Scatchard analysis shows sites with two affinities for both asialoepo and epo. The cells have the same number of receptors for both ligands. But the Kd value for high affinity site with ^{125}I-asialoepo is three-fold lower than that with ^{125}I-epo. This supports the idea that the increased biological activity of asialoepo is due to the change in affinity. The Kd values for the

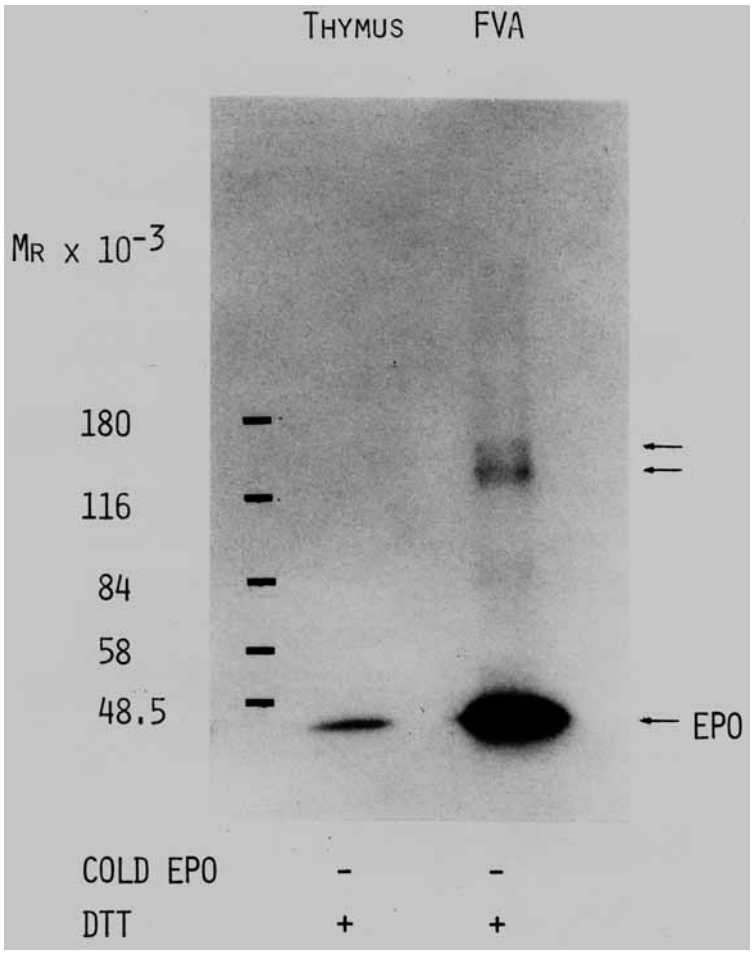

Fig. 6. Autoradiogram showing ^{125}I-epo not cross-linked with mouse thymus cells. Thymus cells or FVA cells were incubated with ^{125}I-epo at 22°C for 2 hours. Cells were then cross-linked with DSS and solubilized. The samples were subjected to SDS-PAGE under reducing conditions, followed by autoradiography. Arrows from top to bottom indicate 142 and 125 kDa bands.

lower affinity binding site are essentially the same for ^{125}I-epo and ^{125}I-asialoepo.

The high and low affinity binding sites in FVA cells are not represented by the two components detected by cross-linking experiments with FVA cells since 201 cells, which have only lower affinity epo receptors, have the same two components (Fig. 5b). D'Andrea et al. (1989) have shown that COS cells transfected with an epo receptor cDNA, derived from a MEL cell line which expresses only low affinity epo receptors, have both high and low affinity receptors. It is possible that an accessory protein in the COS cell can affect the binding of epo to form a receptor complex with increased affinity and that this factor may exist in FVA and COS cells but not in MEL cells.

In cross-linking experiments, ^{125}I-asialoepo was cross-linked to the same two components (based on size) as ^{125}I-epo. This further suggests that asialoepo and epo bind to the same receptors. We assumed that each of the two components bound one ligand, but the true compositions of the radiolabeled bands are unknown. The calculated molecular weight of epo receptor from the MEL cell cDNA is about 55 kDa (D'Andrea et al., 1989) and our data indicate that the molecular weight of the epo receptor is in the range of 55–70 kDa (Dong and Goldwasser, 1990). The relationship between the 88 and 105 kDa cross-linked components and the cloned epo receptor is not at all clear.

Results similar to those reported here on cross-linking of ^{125}I-epo have been reported. The molecular weights of the two components reported vary (94–119 kDa for the heavy component and 78–100 kDa for the light component) (Sawyer et al., 1987a; D'Andrea et al., 1989; Mayeux et al.,

1987; Tojo et al., 1987). However, in most cases the difference in molecular weights of the two components reported by the same group is relatively constant (15–16 kDa) (Sawyer et al., 1987a; Mayeux et al., 1987; Tojo et al., 1987).

In conclusion, our study indicates that the epo receptor has greater affinity for asialoepo then for native epo. This can explain the greater biological activity of asialoepo when compared with native epo in vitro. Asialoepo is cross-linked to the same two receptor components as is epo.

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REFERENCES

Choppin J, Casadevall N, Lacombe C, Wendling F, Goldwasser E, Berger R, Tambourin P, Varet B (1985): Production of erythropoietin by cloned malignant murine erythroid cells. Exp Hematol 13:610–615.

D'Andrea AD, Lodish HF, Wong GG (1989): Expression cloning of the murine erythropoietin receptor. Cell 57:277–285.

Davis JM, Arakawa T, Strickland TW, Yphantis DA (1987): Characterization of recombinant human erythropoietin produced in Chinese hamster ovary cells. Biochemistry 26:2633–2638.

Dong YJ, Goldwasser E (1990): The molecular weight of the erythropoietin receptor. FASEB J. 4:A2142.

Fracker PH, Speck JC Jr (1978): Protein and cell membrane iodination with a sparingly soluble chloroamide, 1,3,4,6,-tetrachloro-3α,6α-diphenylglycoluril. Biochem Biophys Res Commun 80:849–857.

Fukamachi H, Saito T, Tojo A, Kitamura T, Urabe A, Takaku F (1978): Binding of erythropoietin to CFU-e derived from fetal mouse liver cells. Exp Hematol 15:833–837.

Goldwasser E, Kung CKH, Eliason JF (1974): On the mechanism of erythropoietin-induced differentiation. J Biol Chem 249:4202–4206.

Imai N, Higuchi M, Kawamura A, Tomonoh K, Oh-Eda M, Fujiwara M, Shimonaka Y, Ochi N (1990): Physicochemical and biological characterization of asialoerythropoietin: Suppressive effects of sialic acid in the expression of biological activity of human erythropoietin in vitro. Eur J Biochem 194:457–462.

Koury MJ, Sawyer ST, Bondurant MC (1984): Splenic erythroblasts in anemia-inducing Friend disease: A source of cells for studies of erythropoietin-mediated differentiation. J Cell Physiol 121:526–532.

Laemmli UK (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 277:680–685.

Mayeux P, Billat C, Jacquot R (1987): Murine erythroleukemia cells (Friend cells) possess high-affinity binding sites for erythropoietin. FEBS Lett 211:229–233.

Mayeux P, Billat C, Jacquot R (1987a): The erythropoietin receptor of rat erythroid progenitor cells: Characterization and affinity cross-linkage. J Biol Chem 262:13985–13990.

Mufson RA, Gesner TG (1987): Binding and internalization of recombinant human erythropoietin in murine erythroid precursor cells. Blood 69:1485–1490.

Sasaki R, Yanagawa S, Hitomi K, Chiba H (1987): Characterization of erythropoietin receptor of murine erythroid cells. Eur J Biochem 168:43–48.

Sawyer ST, Krantz SB, Goldwasser E (1987): Binding and receptor-mediated endocytosis of erythropoietin in Friend virus-infected erythroid cells. J Biol Chem 262:5554–5562.

Sawyer ST, Krantz SB, Luna J (1987a): Identification of the receptor for erythropoietin by cross-linking to Friend virus-infected erythroid cells. Proc Natl Acad Sci USA 84:3690–3694.

Tambourin P, Casadevall N, Choppin J, Lacombe C, Heard JM, Fichelson S, Wendling F, Varet B (1983): Production of erythropoietin-like activity by a murine erythroleukemia cell line. Proc Natl Acad Sci USA 80:6269–6273.

Todokoro K, Kanazawa S, Amanuma H, Ikawa Y (1987): Specific binding of erythropoietin to its receptor on responsive mouse erythroleukemia cells. Proc Natl Acad Sci USA 84:4126–4130.

Tojo A, Fukamachi H, Kasuga M, Urabe A, Takaku G (1987): Identification of erythropoietin receptors on fetal liver erythroid cells. Biochem Biophys Res Commun 148: 443–448.