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Murine erythroleukaemia cells (Friend cells) possess high-affinity binding sites for erythropoietin

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Murine erythroleukaemia cells represent erythroid precursors blocked near the CFU-E or proerythroblast stage. In contrast to their non-leukaemic equivalents, neither their proliferation nor their differentiation seems to be affected by erythropoietin. However, we show in this paper that both uncommitted and committed, benzidine-positive, cells bind iodinated erythropoietin. The binding is of high affinity ($K_d = 490 \pm 160$ pM) and reversible with a half-life of the complex of 77 ± 19 min. The number of binding sites is low (300–600 per cell). In contrast the haematopoietic non-erythroid cell lines HL 60 and L 1210 and the myeloid-erythroid human cell line K 562 do not exhibit specific binding. If these binding sites represent true hormone receptors, their presence on a permanent cell line should facilitate erythropoietin receptor purification

Erythropoietin binding; (Murine erythroleukemia cell)

1. INTRODUCTION

The study of the mode(s) of action of erythropoietin (Epo) has been hampered until recently by the lack of pure hormone and by the difficulty in isolating homogeneous target cells in sufficiently large numbers. Binding of Epo was first demonstrated with the help of antibodies [1]. Recently, the isolation of the gene of Epo and its use in genetic engineering [2] made the pure hormone available, and techniques were described for obtaining erythroid progenitor cells either from normal erythropoietic organs [3–5], or after treatment of mice with the so-called anemic strain of the virus complex of Friend [6]. Using the latter technique, which induces a transient proliferation of erythroid progenitors still dependent on Epo for their differentiation, Krantz and Goldwasser [7] described a specific binding of tritiated Epo with an apparent equilibrium dissociation constant (K_d) of the order of 5.2 nM and a mean number of 600 receptors per cell. While studying the binding of Epo by erythroid progenitors from rat fetal liver (forthcoming paper), we observed that 'ordinary' murine erythroleukaemia cells (MEL, Friend cells) were also able to bind Epo specifically. These transformed cells are generally considered as being erythroid progenitors (probably CFU-E) blocked in their differentiation and not responsive to Epo but inducible to differentiate by several agents like dimethyl sulphoxide or hexamethylene bisacetamide (HMBA) (reviews on Friend cells in [8–10]). This paper presents some characteristics of Epo binding by Friend cells. A murine lymphoma (L 1210), a human myeloid-erythroid transformed cell line (K 562) and a human myelomonocytic transformed cell line (HL 60) were also tested for Epo binding.

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2. MATERIALS AND METHODS

2.1. *Cell cultures*

MEL cell lines 745 and 707 (clone 17C) were maintained by reseeding every 3–4 days in RPMI 1640 medium (Gibco, Paisley, Scotland) containing 10% (v/v) horse serum. Under these conditions, the cells proliferated exponentially until they reached a density of $3–4 \times 10^6$/ml, with a doubling time of 12–16 h. Differentiation was induced by adding 5 mM HMBA to the cell suspensions at the beginning of the exponential growth: 4 days later, haemoglobinized benzidine-positive cells [11] represented 85–95% of the population.

HL 60 and K 562 cell lines were grown in RPMI 1640 containing 10% (v/v) bovine fetal serum and L 1210 cell line was maintained in Eagle-Dulbecco medium containing 2.5% (v/v) bovine fetal serum.

2.2. *Erythropoietin*

Pure Epo, produced from isolated human Epo gene by Kirin-Amgen, and purchased from Amersham (Amersham, England) had a specific activity of 70000 U/mg. Its labelled derivative, 3-[^{125}I]iodotyrosylerythropoietin, was also purchased from Amersham; 3 batches were used with specific activities at delivery ranging from 730 to 1200 Ci/mmol. In some experiments pig non-radioactive Epo (spec. act. 1010 U/mg protein, CNTS Paris) was also used and gave results completely superimposable to, and therefore combined with, those obtained with the pure human hormone.

2.3. *Measurement of Epo binding*

Cells ($1–8 \times 10^6$) were incubated in 100 μl RPMI 1640 containing 10% (v/v) heat-inactivated bovine fetal serum and graded concentrations of radioactive Epo. At the end of the incubations, the cells were rapidly chilled by addition of 4 ml ice-cold RPMI to each 100 μl sample, and harvested by centrifugation ($180 \times g$, 5 min); they were washed and harvested 3 more times and finally resuspended in 0.5 ml water and their radioactivity measured by γ counting (60% counting efficiency). Non-specific binding was the binding observed after incubations in the presence of concentrations of non-labelled Epo at least 10-times (generally 50-times) those of the radioactive hormone; total binding was the binding observed in the absence of non-labelled hormone and high-affinity ('specific') binding was the calculated difference total minus non-specific binding.

3. RESULTS

3.1. *Friend cell line 745*

3.1.1. Existence of Epo high-affinity binding

Fig.1 presents the results obtained after 2 h incubation at 37°C in the presence of 200 pM (26000 cpm) radioactive Epo and in the presence or absence of 5 nM non-labelled Epo. Bound radioactivities (total, non-specific and specific) were proportional to cell density throughout the range tested ($1–8 \times 10^6$ cells/100 μl); using 10 nM non-labelled Epo instead of 5 nM did not affect significantly non-specific binding. High-affinity binding was also evident after 3 h incubation at 20°C, but not after 5 h incubation at 0°C (not shown).

3.1.2. Kinetics of Epo binding

As shown in fig.2 Epo high-affinity binding at

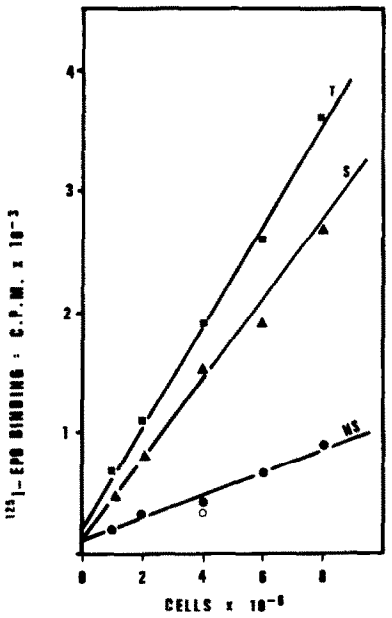

Fig.1. Binding of [^{125}I]Epo to murine erythroleukaemia cells. (■) Total binding (T), (●) non-specific binding (NS) and (▲) high-affinity (specific) binding (S) were determined as described in the text. (○) Non-specific binding measured with 10 nM unlabelled Epo (4×10^6 cells only).

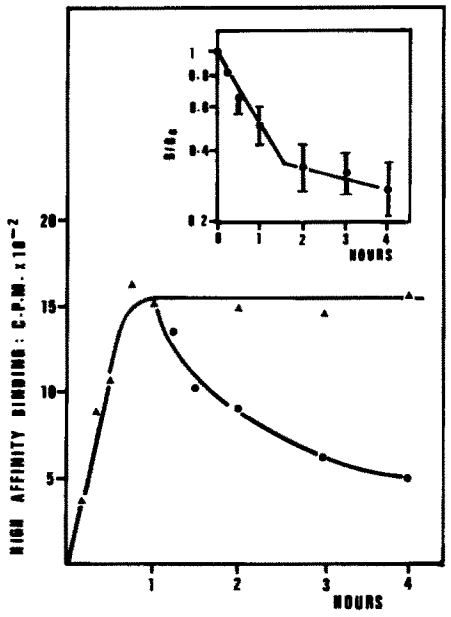

Fig.2. Association and dissociation kinetics of [^{125}I]Epo binding. Results from a typical experiment. 5×10^6 cells were incubated at 37°C with 140 pM [^{125}I]Epo; dissociation was initiated by adding 5 nM unlabelled Epo after 1 h incubation. (Inset) Ratio of specific activity remaining associated with the cells (B)/activity specifically bound by the cells at the beginning of the chase (B_0) was plotted on a logarithmic scale vs time. Each point in the mean of 3 independent experiments and error bars represent the SD.

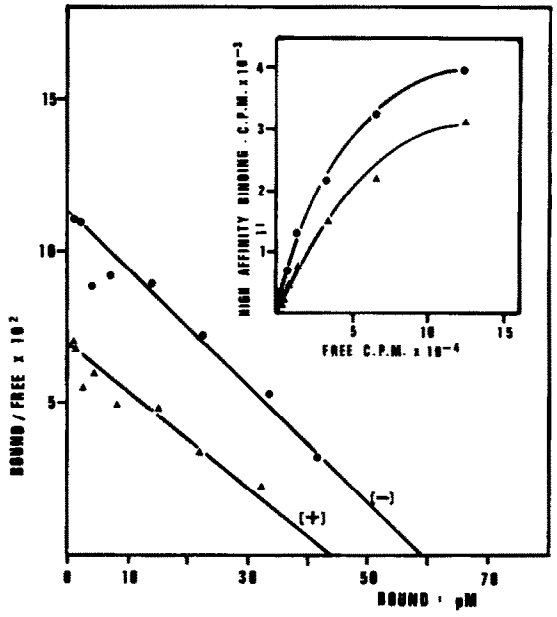

Fig.3. Binding of [^{125}I]Epo to differentiated (+) and undifferentiated (−) murine erythroleukaemia cells as a function of the concentration of [^{125}I]Epo added. Cells (6×10^6 in 100 μl) were incubated at 37°C for 2 h with various concentrations of [^{125}I]Epo. Scatchard analysis of the data. (Inset) Direct representation.

37°C increased during approximately the first 45 min of incubation and remained stable thereafter for at least 4 h. The plateau reflected an equilibrium state of reversible binding since the addition of non-radioactive Epo after 1 h incubation led to a decrease in the specifically bound radioactivity. Expressing this decrease on a logarithmic scale (inset) revealed a first-order dissociation reaction with a half-life of the complex of 77 ± 19 min (mean ± SD of 3 independent experiments). Nevertheless, a small fraction (~30%) of the complex seemed to dissociate more slowly (see section 4).

3.1.3. Characteristics of Epo binding

The apparent equilibrium dissociation constant (K_d) was evaluated on Scatchard representations of high-affinity binding at equilibrium of graded concentrations of labelled Epo (fig.3 shows a typical experiment). 3 independent experiments gave K_d = 490 ± 160 pM (mean ± SD), for non-induced Friend cells of the 745 strain and K_d = 551 ± 168 pM for the same cells after HMBA induction, the number of binding sites per cell being 504 ± 160 and 368 ± 129, respectively. At the end of in-

Table 1

High-affinity binding of [^{125}I]Epo to continuous cell lines of the haematopoietic lineages

Cell lines	Biological characteristics	[^{125}I]Epo specifically bound (cpm × 5 × 10^6 cells)
745	murine erythroleukaemia	1014 ± 61
707 17 C	murine erythroleukaemia	1006 ± 60
L 1210	murine lymphoma	21 ± 29
K 562	human myeloid-erythroid	43 ± 60
HL 60	human myelomonocytic	26 ± 22

Cells in late exponential growth phase were labelled for 2 h with 200 pM [^{125}I]Epo. Labelling was corrected for non-specific binding. Each value is the mean ± SD of 6 determinations

cubation, the medium was submitted to molecular sieve chromatography (Ultrogel AcA 54) or SDS-polyacrylamide gel electrophoresis: in both cases, no significant alteration of Epo during incubation could be detected.

3.2. *Other cell lines*

Friend cells of strain 707 (clone 17 C) also bound Epo with high affinity, but not the non-erythroid L 1210 and HL 60, or K 562 cell lines (table 1).

4. DISCUSSION

As indicated by the Scatchard plots (fig.3), Friend cells bound Epo with high affinity at a single population of sites; the discontinuity observed in the curve representing the time course of dissociation in chase experiments (inset, fig.3) reflects probably a modification of the behaviour of the ligand-receptor complexes (internalization?). As Friend cells are not responsive to Epo and as we did not test for the specificity of binding, we cannot conclude that the high-affinity Epo binding sites are hormone receptors but one may note that: (i) the number of sites per cell is similar to that observed in responsive cells [7], and (ii) non-erythroid cells have no binding sites. The apparent K_d we measured is 10-times smaller than that obtained by Krantz and Goldwasser [7]: this difference might be due to the fact that these authors used a hormone labelled by tritiation of its carbohydrate moiety whereas we used a preparation iodinated on a tyrosyl residue of the peptide backbone. As will be reported in detail elsewhere we also measured Epo high-affinity binding on erythroid progenitors from rat fetal liver and found 500 receptor sites per cell with a K_d of 170 pM. By its nature (heavily glycosylated protein) and its function, Epo is similar to the colony-stimulating factors (CSF) of various haemopoietic lines for which a small number of receptors with very high affinity (K_d between 10^{-9} and 10^{-11} M) seems to be the rule (brief review [12]). Apparently the effects of the neoplastic transformation of erythroid cells on their Epo binding properties are diverse. K 562 cells, which are deeply modified and present granulocytic, monocytic and megacaryocytic properties beside erythroid ones [13] and do not respond to Epo [14], have no receptors. Friend cells, which also do not respond to Epo but exhibit only erythroid potentialities, bind the hormone with high affinity both before and after induced differentiation: the small decrease in the number of binding sites per cell observed after HMBA induction may be related to the decrease in cell size. If the binding sites observed on Friend cells represent hormone receptors the lack of response to Epo may be at the level of signal transduction. Epo was recently reported to stimulate adenylate cyclase activity of the membranes of erythroblasts [15]: it would be interesting to test if the same effect is observed in Friend cells.

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