Editorial

Recent Aspects of Erythropoietin and its Clinical Utility in the Future

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INTRODUCTION

Erythropoietin (EPO) plays an integral role in producing erythrocytes. During the past decade, technological advances like protein purification and gene cloning have made it possible to isolate and characterize various hematopoietic stimulating factors. Many hematopoietins have been detected and their actions on hematopoietic stem cells have been defined. However, the mechanism of action of EPO on the erythroid series via the EPO receptor is still not well understood. The aim of this paper is to review the recent progress in the study of EPO, mainly concerning the EPO receptor and monoclonal antibody against EPO, and to discuss the clinical utility of EPO in the future.

BIOLOGICAL CHARACTERISTICS OF ERYTHROPOIETIN

Erythropoietin (EPO) is one of the glycoprotein hormones. The molecular size has been established to be about 34-40 kilodaltons (kDa) with a specific activity of about 70,000 units per milligram of protein (19). A cDNA encoding EPO has been isolated (20, 21), and a recombinant human EPO with oligosaccharides identical to those on the natural material is now available (22). Its biologic activity in vivo is destroyed by neuraminidase because of its rapid removal from the circulation (1).

EPOs obtained from various mammals are immunologically weakly cross-reactive, but appear to have very similar biochemical and biological properties (2, 3). Renal hypoxia induces the production and release of EPO (4, 5). In humans either hepatocytes or Kupffer cells in the liver produce about 5 to 10% of EPO (6, 7, 8). Extrarenal EPO behaves immunologically and biologically like renal EPO, but its biochemical identity has not as yet been established (9, 10, 11).

The half-life of EPO in circulating blood has been established to be from 1 to 6 hours in experimental animals (12, 13, 14, 15) and up to one or two days in man (16). It has been found that one to four units of EPO are excreted per day at standard hemoglobin concentrations (17). Thus, the liver and kidney are possible sites not only for EPO production but also for EPO catabolism (18, 15).

EPO acts mainly on bone marrow erythroid progenitor cells (EPC) to promote their development into mature erythrocytes (23). The main target cells are the colony-forming units-erythroid (CFU-E). EPO also acts on burst-forming units-erythroid (BFU-E), which are more primitive EPC, to promote their development into CFU-E.
EPO RECEPTOR

Radioiodination of EPO was initially thought to inactivate EPO, but Sawyer et al. have reported that a $^{125}$I-γhEPO ratio of below 0.6 does not reduce its biological activity (24). The behaviour of EPO receptors (EPO-R) cannot be studied in normal hematopoietic tissues because of the extremely small numbers of CFU-E and BFU-E.

In 1984, Koury et al. have reported that mice infected with Friend virus that causes 'anemia' (FVA), show a marked splenic accumulation of erythroid cells. These cells were arrested in differentiation at a stage of development close to the CFU-E and were enriched to provide a population consisting of 95% EPC, with a capacity to respond to EPO in the same manner as do CFU-E (25).

By using fluorescein-labeled EPO or fluorescein-labeled EPO antibodies, Weiss et al. (26) have shown that EPO binds to less than 1% of the bone marrow cells. Because of the low level of EPO responsive cells, it has been difficult to demonstrate the interaction of EPO with its target cells. Purified CFU-E is considered to be a very good tool for investigating the physiological activity of the human EPO-R, but there are two problems; the impurity of the target cells for EPO and the existence of EPO in the culture system. Recently, the EPO-R of human bone marrow cells (27), CFU-E and K562 (28) have also been studied, but such studies are hampered by the impurity of target cells and the existence of intrinsic EPO in the CFU-E system, and by the extremely low number (4-6 sites/cell) of EPO-R. Binding studies of EPO-R using tritiated EPO performed by Krantz and Goldwasser (29) have demonstrated the presence of EPO-R on Friend virus-infected murine spleen cells. Sawyer et al. (30) prepared radiiodinated γhEPO with complete biological activity by using an IODO-GEN method and characterized the EPO-R on the same population of cells as used by Krantz and Goldwasser, but the number of EPO-R they estimated, was an average of 652 ± 269 sites/cell. Thereafter, Fukamachi et al. and other investigators (31, 32) prepared biologically active radiiodinated EPO by using a chloramine-T method. They reported that number of EPO-R on fetal mouse liver cells was 960 ± 57 sites/cell and that on EPO-responsive MEL cells was 543 ± 74 sites/cell (33, 34, 35). These are only same of the many reports concerning the EPO-R.

Table I is a brief summary of the recent findings concerning the EPO-R from 1984 to 1989. Despite the many investigations, only a little is still known about the mechanism of EPO-induced erythroblast proliferation and differentiation. This is chiefly due to the small number of surface EPO-R molecules. Therefore, it is necessary to establish a novel cell line that expresses more EPO-R on its cell surface. Rauscher murine erythroleukemia (R-MEL) cell line has been derived by DeBoth et al. (36) from tumor cells of Rauscher virus-infected DBA mice, and has been shown to synthesize hemoglobin in response to EPO or dimethylsulfoxide (DMSO) induction in plasma clot culture. These cell systems provide a means to investigate a wide variety of questions concerning the biochemistry of erythropoiesis. EPO-induced differentiation results in a specific increase in receptor density on the cell membrane (36, 37). Not only because of the above-mentioned characteristics but also because of the large number of EPO-R (1,600 ± 141 sites/cell) that this cell line expresses, it should serve as a very important research tool in the study of erythroid development and the biochemistry of erythropoiesis.

NEW CELL LINES SECRETING EPO-LIKE ACTIVITY

In 1983, Sytkowski et al. established a continuous human renal carcinoma cell line (GKA) from a patient with the paraneoplastic erythrocytosis syndrome (42). These kinds of cell lines had been reported previously (38, 39, 40), but in none of these reports was it suggested to be associated with erythropoiesis. The cell line is epithelioid, has anchorage development, and secretes EPO activity into the growth medium, consistent with the biological activity of the tumor in vivo. This unique cell line not only permits an investigation of the cellular physiology of this carcinoma but also give us clonal sublines with high EPO-secretory activities (42). Moreover, from above cell line Sytkowski et al. cloned the line GKA which secretes EPO-like activity in up to 6-fold greater amounts than the uncloned line. This activity can stimulate
Recent Aspects of Erythropoietin and its Clinical Utility in the Future—363

Table I  Brief summary of recent aspects about erythropoietin receptor

<table>
<thead>
<tr>
<th>Paper (year)</th>
<th>Author</th>
<th>EPO (RI)</th>
<th>Cell</th>
<th>Receptor(sites/cell) (Kd)</th>
<th>Cross-linking study</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Proc. Natl. Acad. Sci. USA (1984)</td>
<td>Kratz SB Goldwater E</td>
<td>urinary</td>
<td>mice spleen FVA-cells</td>
<td>660(5.2nM) 8(0.06nM)</td>
</tr>
<tr>
<td>2</td>
<td>J. Biol. Chem. (1986)</td>
<td>Sawyer ST et al</td>
<td>urinary</td>
<td>mice spleen FVA-cells MEL cells</td>
<td>300(0.09nM) 500~700(0.57nM)</td>
</tr>
<tr>
<td>4</td>
<td>Proc. Natl. Acad. Sci. USA (1987)</td>
<td>Todokoro K et al</td>
<td>recombinant</td>
<td>MEL cells SKT 6</td>
<td>670(0.15nM) 63kDa 94kDa</td>
</tr>
<tr>
<td>5</td>
<td>Proc. Natl. Acad. Sci. USA (1987)</td>
<td>Sawyer ST et al</td>
<td>recombinant</td>
<td>mice spleen FVA-cells</td>
<td>20%(0.08nM) 80%(0.06nM) 100kDa</td>
</tr>
<tr>
<td>7</td>
<td>Blood (1987)</td>
<td>Muñson RA et al</td>
<td>recombinant</td>
<td>MEL cells</td>
<td>100-200(750pM)</td>
</tr>
<tr>
<td>8</td>
<td>Acta Hemat. Jpn. (1987)</td>
<td>Saito T et al</td>
<td>recombinant</td>
<td>Fetal mouse liver cells</td>
<td>920(0.27nM) 130kDa 145kDa</td>
</tr>
<tr>
<td>9</td>
<td>Development (1987)</td>
<td>Noguchi T et al</td>
<td>recombinant</td>
<td>MEL cells (TSA 8)</td>
<td>500(0.3~0.7nM)</td>
</tr>
<tr>
<td>10</td>
<td>Biochem. Biophys. Res. Commun. (1987)</td>
<td>Sakaguchi M et al</td>
<td>recombinant</td>
<td>DA-1 IL-3 dependent cell</td>
<td>131±23(0.54±0.2nM)</td>
</tr>
<tr>
<td>13</td>
<td>J. Biol. Chem. (1987)</td>
<td>Weiss LT et al</td>
<td>recombinant</td>
<td>REL cells RS-1.5</td>
<td>1200±311(6.43±0.09nM)</td>
</tr>
<tr>
<td>14</td>
<td>Acta Hemat. Jpn. (1988)</td>
<td>Kitamura T et al</td>
<td>recombinant</td>
<td>MEL cells (TSA 8)</td>
<td>90±20(0.25±0.05nM) 590±230(6.1±3.3nM)</td>
</tr>
<tr>
<td>18</td>
<td>Blood (1988)</td>
<td>Fraser JK et al</td>
<td>recombinant</td>
<td>K562</td>
<td>4<del>6(270</del>290pM)</td>
</tr>
<tr>
<td>19</td>
<td>J. Immunol. (1988)</td>
<td>Tsao CJ et al</td>
<td>recombinant</td>
<td>IL-3 dependent MEL cells</td>
<td>50(1.16nM)</td>
</tr>
<tr>
<td>20</td>
<td>Biochem. Biophys. Res. Commun. (1988)</td>
<td>Hitomi K et al</td>
<td>recombinant</td>
<td>JAK-1 Human chronic leukemia cell line</td>
<td>110(60pM) 200(400pM) 120kDa 140kDa</td>
</tr>
<tr>
<td>21</td>
<td>Cancer Res. (1988)</td>
<td>Tojo A et al</td>
<td>recombinant</td>
<td>MEL cells</td>
<td>350<del>650(1.2</del>1.4nM) 130kDa 145kDa</td>
</tr>
<tr>
<td>22</td>
<td>Proc. Natl. Acad. Sci. USA (1988)</td>
<td>Broudy VC et al</td>
<td>recombinant</td>
<td>REL cells RS-1.5</td>
<td>1700(440pM) 95kDa 105kDa</td>
</tr>
<tr>
<td>23</td>
<td>Blood (1989)</td>
<td>Kitamura T et al</td>
<td>recombinant</td>
<td>TF-1</td>
<td>1630(0.4nM) 90kDa 105kDa</td>
</tr>
</tbody>
</table>
the growth and differentiation of CFU-E in plasma clot culture colony assay. However, the secreted EPO-like activity does not cross-react human urinary EPO and heterologous anti-human urinary EPO anti-serum. This suggests that line GKA secretes a new erythropoietic stimulating factor distinct from EPO. This factor may be a precursor to or similar to EPO, an erythropoietic and relatively unprocessed form from the observation that the antigenic determinants are recognized by RIA (43, 44).

**ANTI-PEPTIDE MONOCLONAL ANTIBODY TO HUMAN EPO**

Antibodies reactive with human EPO have been isolated from the serum of rabbits immunized with a 26-amino acid synthetic polypeptide corresponding to the proposed NH terminal sequence of the hormone (45). Inhibition with peptides fragments showed that those antibodies that bound to EPO recognized the domain [8-15], suggesting that this region is exposed on the hormone’s surface (46, 47). This has also been confirmed on immobilized fragments [8-15]. These results have provided information on the tertiary structure of human EPO and have also suggested the usefulness of sequence-specific antibodies in labeling the hormone (48). A site-specific monoclonal antibody to human EPO binds specifically to the peptide $^{125}$I-EPO, and to biologically active EPO. The amino-terminal region of EPO is not involved in receptor binding (49). Other studies have shown that six other regions of the primary sequence have a high probability of being accessible to antibody probes. Antibodies raised against synthetic peptides homologous to five of these residues were found to recognize EPO, which confirmed predictions based upon the relative hydrophilicity of the regions. The effect of these antibodies is reversed by excess peptide, and they exhibit antigen-specific binding (50). These results suggest that the portion of EPO represented by these peptides plays a role in the hormone’s action, probably by forming part of the receptor-binding domain. The precision and power of this site-specific antibody approach have been greatly enhanced by the demonstration that synthetic peptides homologous to specified regions of a protein can be employed as immunogens. The application of monoclonal antibody technology to this area has allowed the preparation of monospecific reagent antibodies directed against selected domains of proteins. Monoclonal antibodies should prove suitable for studies of the cellular localization of erythropoietin biosynthesis, and of its processing after interaction with the receptor. They should also be useful in the immunoaffinity purification of the hormone.

**CLINICAL UTILITY OF EPO IN THE FUTURE**

Recently, recombinant human EPO has become generally available, although EPO was first purified from urine in 1977. Serum levels of EPO have been studied in a variety of clinical disorders for over thirty years using various bioassays, radioimmunoassay was only a sensitive, accurate, and reproducible method which could reliably measure small quantities of EPO and was highly specific. With this method the behaviour of circulating EPO in both healthy volunteers and patients with anemia could be easily and accurately defined.

Renal insufficiency is the classical example of an EPO deficiency state. Now it seems clear that EPO is a very effective and potent drug for increasing and maintaining red cell production in patients with the anemia of end-stage renal disease such as uremia that necessitates chronic hemodialysis.

Rheumatoid arthritis is a prototype inflammatory disorder which is complicated by anemia. Since administration of EPO can correct the anemia associated with rheumatoid arthritis, the low serum EPO levels in this disease appear to be an indicator of a true hormone deficiency state. Rheumatoid arthritis is also associated with increased production of interleukin-1 (IL-1), with increased serum levels as well as synovial fluid levels, and the hematocrit inversely correlates with serum level of IL-1. It has been recently shown that recombinant human EPO can overcome the depression of erythropoiesis in vitro that is produced by IL-1.

HIV infection induces a profound infectious and inflammatory state in which there is an increasing incidence of anemia as the disease progresses. Since anemic AIDS patients with low serum EPO levels respond to administered recombinant EPO with a reduction in transfusion requirements, this situation also represents
in part a hormone deficiency state.

Preliminary studies also indicate that recombinant human EPO may be useful in allowing greater pre-donation of red cell for surgery.

Other anemias in which inappropriately low serum EPO levels have been observed include the anemia of prematurity, the anemia associated with cancer and the anemia associated with myelodysplasias. It seems that recombinant EPO is a effective and potent drug for increasing and maintaining red cell production in some patients with aplastic anemia who have decreased serum EPO levels. Clinical trials of recombinant EPO are currently underway in these anemias and the implication is clear that the clinical assay for serum immunoreactive EPO is useful in evaluating both the etiology on an anemia and the potential of EPO for its correction. But still insufficient information is available yet to indicate whether these patients will benefit from the hormone.

Recombinant human EPO is an extremely specific, potent, and effective agents for increasing erythropoiesis with a minimum of complications. It should prove to be of immense value for treating or limiting severe anemia in a great many patients and improving their well being (51).

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REFERENCES

27) Fraser JK, Lin FK, Berriedale MV: Expression of high affinity receptors for erythropoietin on human bone marrow cells and on the human erythroleukemic cell


