The nitrocellulose membranes were blocked by incubation in 1% polyacrylamide gel electrophoresis and the gels were electroblotted. This was incubated with goat antimouse IgG conjugated to alkaline phosphatase (AP) in TBS for 30 minutes. After washing the membrane, tetrazolium/5-bromo-4-chloro-3-indolyl phosphate as a substrate for AP. Commercial molecular weight markers were used to assess the apparent molecular weights. The staining intensity of the immunoreactive material was detected after the addition of nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate as a substrate for AP. Commercial molecular weight markers were used to assess the apparent molecular weights. The staining intensity of the 34-kD protein corresponding to sEPOR was assessed by using a densitometer (Densitron 20-HR; Jokoh, Tokyo, Japan) and expressed as the relative density, which was calculated as percentage of the staining intensity of a standard sample made by mixing the serum of 5 patients that showed high staining intensity in the screening Western blot. Specificity of the Western blot findings was established by competitive blots, during which incremental amounts of the recombinant EPO ectodomain (a gift from Dr. A. Yoshimura, Kurume University, Fukuoka, Japan) were added in the primary antibody incubation step.

The results of the relative density to estimate sEPOR levels in the sera of normal subjects and anemic patients are shown in Fig. 1. Wide variation of sEPOR levels was observed in normal subjects and anemic patients and no significant difference of sEPOR levels was noted among these subjects. As shown in Table 1, there was no significant correlation between sEPOR levels and various erythroid parameters, including reticulocyte counts, hemoglobin concentration, and serum EPO concentration.

According to Heaney and Golde, soluble receptors play physiologic roles in stabilizing their ligand, modulating concentrations of active ligand, or conferring a responsive phenotype on unresponsive cells by specific binding to their ligand. Unlike these soluble receptors, sEPOR is reported to play a physiologic role other than ligand binding, because it is predicted that secreted sEPOR is lacking most of the region encoded by exon 5 of the EPO gene, which it is suggested to be essential for binding to EPO. Although the physiologic roles of sEPOR remain unclear, the detection of sEPOR in the serum of patients is of clinical interest, because sEPOR is reported to serve as an useful marker reflecting total erythroid progenitor activity. However, our present study was not compatible with the previous finding. Namely, a wide range of sEPOR level was noted in normal volunteers, and relatively high levels of sEPOR were detected in hypoproliferative anemias such as aplastic anemia (AA) and anemia in acute myeloid leukemia (AML), whereas low levels of sEPOR corresponding to those of iron deficiency anemia (IDA) were ob-

![Fig 1. Serum sEPOR levels of controls and anemic patients. The staining intensity of proteins corresponding to sEPOR was expressed as the relative density. Open circles with horizontal bars indicate the mean ± SD.](image)

**Table 1. Correlation Coefficient Between sEPOR Levels and Erythroid Parameters**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>n</th>
<th>EPO</th>
<th>Hemoglobin</th>
<th>Reticulocyte</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>18</td>
<td>0.242</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Patients</td>
<td>38</td>
<td>0.067</td>
<td>0.023</td>
<td>0.020</td>
</tr>
<tr>
<td>IDA</td>
<td>5</td>
<td>0.218</td>
<td>0.131</td>
<td>0.001</td>
</tr>
<tr>
<td>HA</td>
<td>4</td>
<td>0.256</td>
<td>0.150</td>
<td>0.065</td>
</tr>
<tr>
<td>AA</td>
<td>11</td>
<td>0.006</td>
<td>0.227</td>
<td>0.347</td>
</tr>
<tr>
<td>MDS</td>
<td>9</td>
<td>0.282</td>
<td>0.001</td>
<td>0.315</td>
</tr>
<tr>
<td>AML</td>
<td>9</td>
<td>0.475</td>
<td>0.003</td>
<td>0.166</td>
</tr>
<tr>
<td>Total</td>
<td>56</td>
<td>0.051</td>
<td>0.023</td>
<td>0.020</td>
</tr>
</tbody>
</table>

**Abbreviations:** ND, not determined; IDA, iron deficiency anemia; HA, hemolytic anemia; AA, aplastic anemia; MDS, myelodysplastic syndrome; AML, acute myeloid leukemia.

Lack of Relationship Between Soluble Erythropoietin Receptor Levels and Erythroid Parameters in Anemic Patients

To the Editor:

Erythropoietin (EPO) mediates signals through interaction with specific EPO receptors (EPOR). Recent molecular studies showed that alternative splicing of the EPO gene produced three types of EPOR: the full-length EPOR (EPOR-F), the truncated form of EPOR (EPOR-T), and the soluble EPOR (sEPOR).\(^1,^2\) EPOR-T, the most prevalent form of EPOR in early stage erythroid progenitors, can transduce a mitogenic signal, whereas EPOR-F, the most prevalent form of late-stage progenitors, can transduce a signal to prevent programmed cell death. The biologic significance of sEPOR is not entirely clear. In a previous issue of Blood, Baynes et al\(^7\) reported that the presence of sEPOR was highly correlated with enhanced erythropoiesis. This finding indicates that the measurement of sEPOR may be helpful in the clinical assessment of erythropoiesis in a variety of anemias.

In the present study, to determine whether sEPOR may serve as an useful indicator to estimate the erythropoiesis in anemic patients, we analyzed the levels of sEPOR in the serum of healthy volunteers as well as of anemic patients of various causes and investigated the relationship between sEPOR levels and erythroid parameters, including serum titers of erythropoietin.

The levels of sEPOR were analyzed by the Western blot described elsewhere.\(^1\) In brief, sera were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and the gels were electrophotoblot. The nitrocellulose membranes were blocked by incubation in 1% bovine serum albumin-Tris-buffered saline (TBS) for 2 hours and incubated with monoclonal antibody against human EPO ectodomain (mh2ER/7.9.2; a generous gift from Genetics Institute, Inc, Cambridge, MA) in TBS for 2 hours. After washing, the membrane was incubated with goat antimouse IgG conjugated to alkaline phosphatase (AF) in TBS for 30 minutes. After washing the membrane, the staining intensity of proteins corresponding to sEPOR was expressed as the relative density. Open circles with horizontal bars indicate the mean ± SD.
served in hyperproliferative anemia such as hemolytic anemia (HA). Furthermore, sEPOR levels of control subjects and anemic patients had no correlation with erythroid parameters; therefore, we could not find relationship between sEPOR levels and erythropoiesis. The discrepancy between the findings of the previous report and those of the current study cannot be explained at present. Although further studies will be required, our present study suggests that secreted sEPOR plays a physiologic role other than erythropoiesis, but the wide range of sEPOR levels may indicate important roles in the regulation of sEPOR expression. Thus, we conclude that it is necessary to determine the regulation mechanism for generating sEPOR in erythropoiesis.

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Izumi Hirasawa
Mariko Murayoshi
Kunitake Hirashima

Response: Two Serum Forms of the Erythropoietin Receptor

We have previously reported that a 32-kD protein is detected in human serum in proportion to erythropoietic activity using a Western blot based assay with a polyclonal antibody raised to the amino-terminal peptide sequence APPNLDPKFKESK of the erythropoietin receptor (EPOR). We have recently reported that this 32-kD protein is not reactive with polyclonal antibodies produced to the peptide sequences EPSFGFWSAWSEP in the region of the WSAWSE motif and (C)AWSEPVSLLTPSDLDP at the carboxyterminus of the EPOR ectodomain. We have developed and reported a polyclonal enzyme-linked immunoassay (EIA) using polyclonal antibodies raised against a purified recombinant soluble EPOR ectodomain. This assay detected EPOR in human serum, but the level correlated poorly with aminoterminal peptide antibody reactive serum EPOR and erythropoietic activity. These data are in complete agreement with the findings reported by Yoshida et al in their letter. EIA strongly positive sera that were not immunoreactive with aminoterminal peptide antibody showed a 29-kD immunoreactive protein in Western blots using EPSFGFWSAWSEP and (C)AWSEPVSLLTPSDLDP peptide antibodies. These data are consistent with the finding reported by Yoshida et al that a 32-kD moiety being reactive with the aminoterminal peptide antibody but nonreactive with the other two peptide antibodies in and about the WSAWSE region is highly suggestive of the alternatively spliced, truncated form of EPOR termed sEPOR in the letter of Yoshida et al. The 29-kD protein that is nonreactive with the aminoterminal peptide antibody yet reactive with the other two peptide antibodies and the antibody produced to the purified recombinant EPOR ectodomain is suggestive of a clipped ectodomain that has been subjected to proteolytic cleavage at both ends.

We have also recently reported the subcloning and expression in a mammalian expression system of the alternatively spliced, potentially secreted EPOR, referred to in the letter of Yoshida et al as sEPOR. The protein does appear to be secreted. However, of interest is the finding that only one of a number of monoclonal antibodies raised to the EPOR ectodomain is reactive with this alternatively spliced form. More specifically, monoclonal antibody 7.9.2 reported by Yoshida et al is nonreactive with sEPOR (unpublished observation). Furthermore, in unpublished data from our group, an EIA using 7.9.2 monoclonal and polyclonal anti-EPOR ectodomain antibodies obtained results consistent with our polyclonal EIA data. In summary, the data of Yoshida et al are completely explicable and consistent with our published data. However, their interpretation that sEPOR does not correlate with erythropoiesis is in all likelihood incorrect, because monoclonal antibody 7.9.2 does not appear, in our studies, to react with our subcloned and expressed sEPOR. The clinically informative 32-kD protein is consistent with sEPOR.

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REFERENCES

Lack of relationship between soluble erythropoietin receptor levels and erythroid parameters in anemic patients [letter; comment]

S Yoshida, M Bessho, K Sakate, I Hirasawa, M Murayoshi and K Hirashima