Early pregnancy factor (EPF) is a pregnancy-associated protein detected in the maternal serum by using the rosette inhibition assay and by evaluating the suppression of adoptive transfer of contact sensitivity. Because of its inhibitory effect on the functional activity of immunocompetent cells, EPF is thought to be involved in immunoregulation of the maternal immune system during early pregnancy. EPF was purified six million-fold from the serum of pregnant women between 5 and 12 weeks of gestation. The specific activity of purified EPF was approximately $8 \times 10^8$ units/mg. The purification scheme involved sequential DEAE-cellulose chromatography, S-Sepharose chromato- graphy, concanavalin A-Sepharose chromatography, heparin-Sepharose chromatography, Mono S fast protein liquid chromatography, and preparative sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The purified protein has an apparent molecular weight of 21,500 as analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and 28,000 by gel permeation high pressure liquid chromatography. The isoelectric point of purified EPF moiety is 6.5. The biological activity was susceptible to the proteolytic enzyme trypsin, acidic pH conditions, organic solvents, and sodium dodecyl sulfate, but stable to heat treatment at 56°C for 30 min and the reducing agent dithiothreitol. The biological and physicochemical properties of EPF appear to be distinct from other pregnancy-associated and immunoregulatory proteins.

The mechanism of maternal tolerance to the semi-allogeneic fetus has been debated for years without any agreement in the literature (1-4). It is speculated that a complex of immunoregulatory processes may function in the mother at different times of gestation, to enable the survival of the fetus. Evidence for the presence of immunomodulating substances in the maternal blood during pregnancy emerges from the observation that serum from pregnant animals is capable of inhibiting lymphocyte responses in several assay systems (5-7). These modulating agents mediating the maternal immune response during pregnancy are categorized as soluble serum factors (8), gestational hormones (9), placental proteins (10), and factors released from immunocompetent cells.

Early pregnancy factor (EPF) is one such pregnancy-associated immunosuppressive protein released into the maternal circulation within hours after fertilization. EPF was first described by Morton et al. (11) as an activity detected in pregnancy sera by using an immunological assay called the rosette inhibition test. This assay was originally devised to evaluate the immunosuppressive potency of anti-lymphocyte serum, which contains antibodies directed against lymphocyte surface antigens (12). When mononuclear cells are exposed to heterologous red blood cells, a small sub-population of lymphocytes bind to red blood cells to form spontaneous rosettes (13). The formation of these rosettes is inhibited by prior incubation of lymphocytes with ALS, in a dose-dependent manner. If, in addition, the lymphocytes are incubated with pregnancy serum prior to incubation with ALS, the concentration of ALS required to inhibit rosette formation is significantly lowered. The pregnancy-associated serum constituent causing this enhancement in the rosette inhibiting capacity of ALS has been termed EPF. By using this assay, EPF activity has been detected very early in gestation, in the serum of most mammalian species tested so far (14, 15).

Since EPF inhibits rosette formation, it is thought to interact with T-lymphocytes and suppress cellular immune responses. EPF has also been reported to inhibit the adoptive transfer of contact sensitivity to the soluble antigen, trinitrofluorobenzene (16). Suppressive effects of EPF on cellular immune responses have also been demonstrated in some other immunological assay systems. A recent study has shown that EPF inhibits, in a dose-dependent manner, the recognition phase in allogeneic mixed lymphocyte responses (17). EPF also inhibits the early expression of cell surface membrane IgG (18). These observations support the idea that EPF has an inhibitory effect on the functional reactivity of immunocompetent cells. This fact, taken together with the observation that EPF is released very early in gestation, has led to the proposal that EPF participates in the regulation of the maternal immune system at very early stages of pregnancy. This is further supported by observations that embryo loss arising from spontaneous or therapeutic abortions is coincident with the absence of EPF activity (19) and that administration of anti-EPF antibody in the mice, before implantation, decreased the number of implantation sites as well as neoneats in the treated animals (17).

So far, experimental data on EPF have been obtained using crude or partially purified preparations of the molecule(s). In order to better understand the biology of EPF, it is necessary
to obtain the protein purified to homogeneity. Earlier attempts by several workers to purify and characterize the EPF molecule(s) from the serum of pregnant sheep (20–22), pigs (23), humans (24), as well as conditioned medium from mouse embryos cultured in vitro (25), have met with limited success. This could be partly due to heterogeneity in the molecular size of the protein, as reported in some species (21, 22) and also due to the fact that only trace amounts of the active protein are present in the maternal serum. In this report, we describe the purification of EPF from pooled pregnancy sera derived from pregnant women between 5 and 12 weeks of gestation. The physicochemical characteristics of the EPF moiety as well as a comparison of EPF with other known pregnancy-associated proteins and immunosuppressive proteins is also discussed.

EXPERIMENTAL PROCEDURES

Materials—DEAE-52 cellulose (pre-swollen) was purchased from Whatman; S-Sepharose (fast flow), heparin-Sepharose, concanavalin A-Sepharose, Mono S (HR 5/5), and Mono P (HR 5/5) columns, Polybuffer 10 and Ficol-Hypaque were obtained from Pharmacia Fine Chemicals; pre-stained molecular weight markers were from Bethesda Research Laboratory; polyethylene sorbitan monolaurate (Tween 20), dithiothreitol, heparin, bovine serum albumin, and Hanks’ balanced salt solution (HBSS) were purchased from Sigma. Sheep red blood cells were obtained from Colorado Serum Co.

Collection of Pregnancy Serum—Blood was collected from women within the 6th–12th gestational week of normal pregnancy. All patients were healthy subjects. The serum was separated by centrifugation and stored at -20°C. Prior to processing, the serum samples from various subjects were pooled and inactivated at 56°C for 30 min.

Measurement of EPF Activity—EPF activity in pooled pregnancy sera and fractions thereof were measured using the rosette inhibition assay as described by Morton et al. (26). 25 ml of pooled serum collected from healthy nonpregnant donors and mononuclear cells separated by ficoll gradient centrifugation. The lymphocytes were washed three times and resuspended to a concentration of 10^6 cells/ml in HBSS. A typical assay run consisted of four test samples and two controls. The cells were preincubated with test and control sera for 30 min at 37°C, followed by two washes with HBSS. An initial starting dilution (1:1000) of ALS in HBSS was serially diluted into nine tubes, to give dilution ranging from 1000- to 512,000-fold. The two tubes which contained no ALS were the internal controls. The ALS was heat inactivated at 56°C for 30 min and absorbed with human and sheep red blood cells (10^7/ml) prior to use in the assay. Cell washing, 100 µl of the preincubated lymphocytes were added to each tube containing 250 µl of the serially diluted ALS and 50 µl of guinea pig serum (absorbed with human and sheep red blood cells and diluted 1:5 with HBSS just before use). The tubes were then incubated for 90 min at 37°C. After cooling to room temperature, 100 µl of sheep red blood cells (10^6 cells/ml) were added to each tube followed by centrifugation at 200 x g for 5 min. Subsequently, cell pellets were resuspended on a wheel rotating at 10 rpm for 5 min before being spread on a hemocytometer. The rosettes were visualized and counted with a phase-contrast microscope.

RESULTS

In order to investigate an optimum and convenient source for isolation of EPF, its presence in pooled human pregnancy sera, human pregnancy urine, and conditioned media from BeWo (human choriocarcinoma cell line) cell line (31) was examined. The results indicated that the specific activity in human pregnancy sera (100–150 units/mg) was significantly higher compared to the activity detected in pregnancy urine (0.09 units/mg) and cell conditioned medium (1.0 units/mg). Also, it has been reported the EPF levels in the serum are significantly higher compared to the activity detected in pregnancy urine (0.09 units/mg) and cell conditioned medium (1.0 units/mg). Also, it has been reported the EPF levels in the serum are
highest during early stages of pregnancy (29, 30). Hence, for our study, serum derived from pregnant women (5–12-weeks gestation) was used as the source of starting material.

The pooled pregnancy serum (210 ml; ~16 g of protein) had EPF activity of 150 units/mg. This pool was dialyzed against Buffer A and applied to a DEAE-52 (anion exchange) cellulose column. Based on the results of some earlier experiments (24), the column fractions were combined into two pools: the unbound protein fraction comprising the flow-through and wash and the bound protein fraction eluted with 1 M sodium chloride in Buffer A. The unbound protein fraction containing less than 10% of the serum proteins had all of the EPF activity (Table I), providing a 14-fold purification and quantitative recovery of the active molecule(s).

The effluent from the DEAE-52 column was then applied to a S-Sepharose (cation exchange) column. Under these conditions, the flow-through and wash contained 80% of the proteins loaded, but did not have any activity. EPF activity, in fact, bound quantitatively to the S-Sepharose column and could be eluted with 0.1 M sodium chloride in Buffer A. This eluate contained only 3% of the total protein applied to the column, with approximately 80% recovery of EPF activity, giving 25-fold purification at this stage.

The active fraction from the S-Sepharose column was then fractionated on a Concanavalin A-Sepharose column equilibrated with Buffer B. The effluent comprising the flow-through and wash contained all of the EPF activity and approximately 40% of the protein applied to the column. This fraction was dialyzed against 50 mM Tris containing 0.1 M KCl and 1 mM sodium azide, pH 7.0, concentrated, and applied to a heparin-Sepharose column equilibrated with the same buffer. The column was eluted with a linear gradient of 0.1 M to 1.0 M KCl in 50 mM Tris. EPF activity was associated with the bound protein fraction, eluting at a salt concentration of 0.45 M KCl. This step provided greater than 15-fold increase in the purification of EPF with 85% recovery of the biological activity. Analytical SDS-PAGE of this active fraction revealed the presence of various other major bands in addition to the presence of some bands in the region of Mr 20,000 (data not shown).

Further purification was achieved by chromatography of the active fraction by Mono S FPLC (Fig. 1). It can be seen that application of a shallow salt gradient resulted not only in the elution of EPF activity, but also its separation from the bulk of the contaminating proteins. The protein content of the Mono S-purified, EPF-active fraction was determined on the basis of intensity of silver-stained bands obtained on SDS-PAGE, by comparing with the staining intensity observed for recombinant tumor necrosis factor-α. This step provided a further 6-fold increase in purification with 80% recovery of EPF activity.

The above sample was subjected to preparative sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and this resulted in the resolution of a single protein of molecular mass 21,500 daltons that coincided with biological activity (Fig. 2). Although EPF activity is susceptible to SDS treatment (Table II), detectable quantities of the active protein could be eluted from the gel slices cut in the region of molecular mass 20,000 daltons. No activity was detected in the protein eluate of gel slices cut from the remaining region of the SDS-gel track. The eluate from the SDS-gel slices had only 20% of the total applied biological activity and provided a 67-fold increase in purification of the active protein. From 210 ml of human pregnancy sera containing 16 g of protein, approximately 250 ng of the active protein was obtained. This provided an overall 6 × 10⁶-fold purification of EPF, with only 11% recovery of the biological activity.

Characterization of Purified EPF—The specific activity of purified EPF was estimated to be approximately 8 × 10⁶ units/mg of protein. However, if correction for loss of activity due to SDS treatment were taken into account, a higher specific activity of EPF would be obtained.

The molecular weight of purified EPF was analyzed by SDS-PAGE and gel permeation chromatography. EPF purified by preparative SDS-PAGE was assessed on analytical SDS-PAGE under the reducing and nonreducing conditions. A single major protein band of an apparent molecular mass of 21,500 daltons was observed (Fig. 2, inset). The molecular weight of EPF was also determined by TSK-HPLC under non-denaturing conditions. A major peak of activity with a retention time of 36 min, corresponding to a molecular weight of 28,000, was observed. A total of 60% of the applied activity was recovered in this peak (Fig. 3). After storage at 4 °C (2–4 weeks), as much as 50% of the total applied activity was observed at higher molecular mass region of 43,000 daltons (data not shown).

The isoelectric point of Mono Q FPLC-purified EPF was determined by chromatofocusing on a Mono P column attached to a FPLC system. A single peak of EPF activity was observed at pH 6.5 with a total recovery of 90% (Fig. 4).

Preliminary studies on the stability of the partially purified EPF molecule(s) are presented in Table II. EPF activity is labile to proteolytic enzymes such as trypsin, overnight exposure to acidic buffer, pH 2.0, exposure to 0.1% SDS and 50% propanol, respectively, and exposure to temperatures higher than 70 °C. However, EPF activity was not destroyed after treatment with 1 mM dithiothreitol and exposure to 56 °C for 30 min.

Several proteins which are known to be associated with pregnancy or implicated with immunosuppressive properties were analyzed for EPF activity in the rosette inhibition assay.

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Final volumes</th>
<th>Total protein</th>
<th>Total activity</th>
<th>Relative specific activity</th>
<th>Purification</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starting material</td>
<td>210 ml</td>
<td>15,540 mg</td>
<td>2.1 × 10⁶ units</td>
<td>135 units/mg</td>
<td>100</td>
<td>%</td>
</tr>
<tr>
<td>DEAE-cellulose chromatography</td>
<td>1,050 ml</td>
<td>1,365 mg</td>
<td>2.7 × 10⁶</td>
<td>1,941 units/mg</td>
<td>14</td>
<td>126</td>
</tr>
<tr>
<td>S-Sepharose chromatography</td>
<td>430 ml</td>
<td>34 mg</td>
<td>1.7 × 10⁶</td>
<td>49,411 units/mg</td>
<td>366</td>
<td>80</td>
</tr>
<tr>
<td>Concanavalin A Sepharose chromatography</td>
<td>810 ml</td>
<td>13 mg</td>
<td>1.7 × 10⁶</td>
<td>0.13 × 10⁶ units/mg</td>
<td>863</td>
<td>79</td>
</tr>
<tr>
<td>Heparin-Sepharose chromatography</td>
<td>24 ml</td>
<td>0.7 mg</td>
<td>1.4 × 10⁶</td>
<td>2.0 × 10⁶ units/mg</td>
<td>14,815</td>
<td>67</td>
</tr>
<tr>
<td>Mono S fast protein liquid chromatography</td>
<td>2.8 ml</td>
<td>0.1 mg</td>
<td>1.2 × 10⁶</td>
<td>12.0 × 10⁶ units/mg</td>
<td>88,889</td>
<td>57</td>
</tr>
<tr>
<td>Preparative SDS-PAGE</td>
<td>1.0 ml</td>
<td>0.00025 mg</td>
<td>0.2 × 10⁶</td>
<td>800 × 10⁶ units/mg</td>
<td>6 × 10⁶</td>
<td>11</td>
</tr>
</tbody>
</table>

*Approximate estimates of proteins based on their silver-staining intensity.

*This value is not corrected for loss of activity due to treatment of sample with sodium dodecyl sulfate.
The present study details a purification protocol for isolating EPF from pooled human pregnancy sera. Isolation and characterization of the EPF moiety was based solely on the rosette inhibition assay. Fractionation of human serum on DEAE-cellulose results in 90% of the serum proteins being bound to the column with minimal binding of EPF (Table I). This observation is in accordance with earlier reports on human and sheep EPF (20, 24). An excess of the total biological activity applied to the column was recovered in this step.

FIG. 1. Elution profile after application of EPF active fraction on the Mono S cation exchange column (FPLC) using 20 mM sodium phosphate buffer, pH 8.0; flow rate, 1 ml/min; chart speed, 2 mm/min. ---, conductivity profile; ■, presence of EPF activity; —, absorbance profile.

FIG. 2. Profile of EPF activity and silver-stained gel pattern (inset) after SDS-polyacrylamide gel electrophoresis. 12.5% polyacrylamide gel (0.75 mm thick) containing 0.1% SDS was used. After electrophoresis, the sample gel was sliced into 2-mm sections, protein eluted in 50 mM NH4HCO3, and assayed for EPF activity. The position of the various molecular weight standards is indicated.

The results, as shown in Table III, indicate that none of these proteins had any EPF activity even at high concentrations.

**DISCUSSION**

A majority of the biochemically characterized pregnancy-associated proteins and hormones, attributed with immunosuppressive properties, are glycoproteins with varying concentrations of carbohydrate content (19). Notable among these are human chorionic gonadotropin (hCG), α-fetoprotein, pregnancy-associated β2-macroglobulin, pregnancy-specific β1 glycoprotein, and pregnancy-associated plasma protein A. Lack of binding of the EPF moiety to Concanavalin A distinguishes the EPF molecule(s) from these known proteins. However, the presence of trace quantities of sugar in the active fraction cannot be ruled out.

The purified protein, obtained after using the presently described purification scheme, has an apparent molecular mass of 21,500 daltons as analyzed by SDS-PAGE and 28,000 daltons by gel filtration HPLC. The difference in molecular weight of EPF by SDS-PAGE and by gel filtration does not

---

**TABLE II**

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Duration</th>
<th>Activity remaining</th>
<th>% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>10% trypsin (w/w)</td>
<td>Overnight</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>pH 2.0</td>
<td>Overnight</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>0.1% sodium dodecyl sulfate</td>
<td>2 h</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>0.1% trifluoroacetic acid + 50% propanol</td>
<td>2 h</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Dithiothreitol (1 mM)</td>
<td>Overnight</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>30 min</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>56 °C</td>
<td>20 min</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>70 °C</td>
<td>20 min</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>80 °C</td>
<td>20 min</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>90 °C</td>
<td>20 min</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>70% (NH4)2SO4 pellet</td>
<td></td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Iodination</td>
<td></td>
<td>&lt;1</td>
<td></td>
</tr>
</tbody>
</table>

*For trypsin treatment, a parallel control sample (without EPF activity) was included. After overnight incubation at 37 °C, enzyme reaction was stopped by adding 10% of nonpregnant human serum. After treatment of purified EPF active fraction with acid (HCl), SDS, trifluoroacetic acid + propanol, dithiothreitol, and (NH4)2SO4, at room temperature, the samples were dialyzed against phosphate-buffered saline and then analyzed for EPF activity. Iodination of EPF was done by the iodojen method (42).
Purification of Human Early Pregnancy Factor

**Table III**

<table>
<thead>
<tr>
<th>Proteins*</th>
<th>Source</th>
<th>Amount of protein tested in bioassay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relaxin</td>
<td>Genentech</td>
<td>1.0</td>
</tr>
<tr>
<td>Inhibin</td>
<td>Genentech</td>
<td>1.0</td>
</tr>
<tr>
<td>TGF-β</td>
<td>Genentech</td>
<td>1.0</td>
</tr>
<tr>
<td>β2-Microglobulin</td>
<td>Sigma</td>
<td>10</td>
</tr>
<tr>
<td>β-Lactoglobulin</td>
<td>Dr. K. Brew (Miami University)</td>
<td>15</td>
</tr>
<tr>
<td>Aphrodin</td>
<td>Genentech</td>
<td>1.0</td>
</tr>
<tr>
<td>Uteroglobulin</td>
<td>Dr. A. B. Mukherjee (NIH)</td>
<td>10</td>
</tr>
<tr>
<td>Interferon-α</td>
<td>Hoffman-La Roche</td>
<td>0.1</td>
</tr>
<tr>
<td>Interferon-β</td>
<td>Hoffman-La Roche</td>
<td>1.0</td>
</tr>
<tr>
<td>Interferon-γ</td>
<td>Genentech</td>
<td>1.0</td>
</tr>
</tbody>
</table>

*All proteins except β2-microglobulin were greater than 96% pure.

TGF-β, transforming growth factor-β.

appear to be due to the SDS-PAGE-purified sample (partially denatured) used for gel filtration, since similar results were obtained when EPF sample after the Mono P FPLC step was applied on a TSK column. The slightly higher molecular weight of EPF, observed after fractionation of the purified active fraction on the TSK column, corresponds to the peak active fraction recovered from the column and is only an approximate indicator of molecular size. However, these results indicate that EPF, both under native and denaturing conditions, exists as a monomer with almost similar molecular weights. By the criteria of co-migration of protein and elution of biological activity on SDS-PAGE, EPF isolated here appears to be electrophoretically homogeneous. EPF was purified six-million-fold to a specific activity of 8 \( \times 10^5 \) units/mg. In fact, the purified EPF molecule significantly inhibits rosette formation even in the absence of ALS (data not shown).

Partially purified sheep (32) and mouse (25) EPF molecules have also been reported to have activity at extremely low concentrations (0.1 and 20 pg, respectively) of the protein.

As mentioned above, EPF activity has also been characterized in other species including mouse, pig, and sheep (21-25). The physicochemical characteristics of human EPF differ slightly from the various EPF forms isolated from other sources. Earlier work on sheep EPF (32) indicated the existence of three different forms of EPF with apparent molecular weights of 20,000, 50,000, and 250,000. Recent observations by the same authors have indicated the EPF molecule to exist in two different forms with molecular weights of 20,000 and 67,000, respectively. It is not yet known whether the higher molecular weight form is an oligomeric form of the 20,000 molecule (21). Both, the 20,000 and 67,000 forms of sheep EPF are resistant to proteolytic enzymes without prior reduction (21). EPF purified from ovine placental extract has an apparent molecular weight of 12,000 (22). Mouse EPF, isolated from conditioned medium of *in vitro* cultured embryos, has an apparent molecular weight of 21,000 with an isoelectric point of 6.8, which was apparently similar to that observed for human EPF purified here. However, under denaturing conditions, this purified mouse EPF was reported to dissociate into three subunits with apparent molecular weights of 10,500, 7,200, and 3,500, respectively, and these subunits individually were devoid of EPF activity. In contrast, human EPF appears to be monomeric with apparently the same molecular weights under denaturing and non-denaturing conditions. Occasionally, only on storage of the purified fraction, oligomeric form(s) of the human EPF molecule were observed.

There are several pregnancy-associated proteins which either exhibit immunosuppressive, physicochemical, or physiological profile similar to that observed for EPF. These include human chorionic gonadotropin (hCG), α-fetoprotein, human placental lactogen, inhibin, relaxin, placental-secreted proteins like placental protein 14, pregnancy-associated α2-macroglobulin, pregnancy-associated plasma protein A and pregnancy-specific β1 glycoprotein. Although most of these proteins have been implicated with immunosuppressive properties (10), the molecular nature and physiological profile of EPF during pregnancy distinguishes it from AFP, pregnancy-associated α2-macroglobulin, pregnancy-associated plasma protein A, and pregnancy-specific β1 glycoprotein. EPF and human placental lactogen have almost similar molecular size, but the latter does not have any immunosuppressive activity at physiological concentrations.

Human inhibin and relaxin, which are reported to be present in maternal serum at early stages of pregnancy (33, 34), did not have EPF activity. Uteroglobin, the rabbit analogue of human pregnancy-associated endometrial α2-globulin, is reported to be an immunosuppressive pregnancy protein (35) but was devoid of EPF activity (Table III). EPF and hCG have an almost similar physiological profile during pregnancy in that both these proteins are secreted early in gestation with concentration reaching a peak value during the first trimester and then declining to almost undetectable levels during the third trimester of pregnancy. However, hCG differs from EPF in its molecular size and from the observation that purified hCG does not have any EPF activity. Also, experiments indicating that pregnancy serum having undetectable levels of hCG (<5 mIU/ml) still exhibits EPF activity (36) further support the molecular distinctness of EPF. Recently, a novel pregnancy-associated bovine protein, having structural homology to α-interferon, is suggested to have an immunosuppressive role during pregnancy (37). Also, since the interferons, especially, have been attributed with immunoregulatory properties (38), it was interesting to examine if any of the interferons exhibited EPF activity. Our studies indicated that all the three interferons i.e. α-interferon, β-interferon, and γ-interferon were devoid of EPF activity in the rosette inhibition assay. Conversely, purified human EPF lacked any antiviral activity (data not shown).

The basis of how the rosette-inhibiting property of EPF relates to suppression in the reactivity of immunocompetent T-lymphocytes is not known. The ability to form sheep red blood cells rosettes was the conventional method of detection and enumeration of T-lymphocytes. Co-capping and blocking
experiments have shown that this property of the T-lymphocytes is associated with the CD2 molecule (molecular mass 50,000 daltons), also known as the "E receptor." CD2 antigen is a nonpolymorphic molecule and has been viewed both as a "negative signal receptor" and as a receptor for "an alternative pathway of T cell activation on the basis of inhibitory or stimulatory effects found with monoclonal anti-CD2 antibodies (39, 40)." Because the inhibitory and stimulatory anti-CD2 antibodies represent different epitopes of the CD2 antigen and because the stimulatory effects were found with pairwise combination of antibodies that address different CD2 epitopes (41), it is not known whether the differential effects are due to epitope-related signal (negative or positive) generation or to as yet unidentified mechanisms. Since EPF enhances the rosetting-inhibiting capacity of not only the polyclonal ALS, but also of the monoclonal antibodies, anti-Hu-Lyt-3 and OKT11 (monoclonal antibodies specific for CD2 antigen on T-lymphocytes), it is tempting to speculate that EPF may be a natural ligand with affinity for the CD2 receptor, and interaction between the two molecules may contribute to the generation of some kind of obligatory cell signals. However, it still remains to be demonstrated that EPF specifically binds to the CD2 receptor, but with the availability of purified EPF; this should be possible and thus aid in elucidating the precise mechanism of EPF activity.

Acknowledgments—We would like to thank Drs. Mike Kray and Bob Smith of the Presbyterian Hospital in San Francisco; Drs. G. Weiss and Laura Goldsmith of the UMDNJ, Newark, New Jersey for providing us with human pregnancy serum; Dr. A. B. Mukherjee of the National Institutes of Health for the supply of uteroglobulin; Dr. Keith Brew of Miami University for the supply of β-lactoglobulin; Hoffman La Roche for gifts of α and β interferons; Drs. Karoly Nicolis and Paul Johnston of Genentech for the supply of human inhibin and relaxin, respectively; Becker Howes for technical assistance and Dr. Ramani Aiyer for critically reading this manuscript.

REFERENCES