Early Pregnancy Factor: An Unresolved Molecule

Early pregnancy factor (EPF) was first identified in 1974 as a factor associated with pregnancy serum which was active in the rosette inhibition test (1). During the ensuing 22 years attempts to isolate and purify EPF have proven unsuccessful despite many reports of serum fractions or nonspecified proteins having EPF-like activity. Despite the failure to isolate a molecule with specific EPF activity, a number of properties have been described: under normal physiologic conditions it is detected only during pregnancy; its synthesis corresponds to the initiation of syngamy; serum EPF activity is strongly associated with the low molecular weight protein fraction of the serum; the initial site of synthesis is the ovary and subsequently at the blastocyst stage; and it is detectable in all mammals investigated to date, i.e., mice (1), humans (2), sheep (3), cattle (4), red deer (5), and elephants (Morton, personal communication). Due to the ubiquitous nature of EPF it is considered to be important in the establishment of a successful pregnancy, possibly as an immunosuppressive agent and as a growth factor.

Possible candidate proteins for EPF such as human chorionic gonadotrophin, follicle stimulating hormone, luteinizing hormone, and thyroid-stimulating hormone have all been tested for EPF-like activity in the rosette inhibition test but failed to demonstrate EPF activity (2). More recently two further proteins have been proposed to be EPF, thioredoxin (6) and chaperonin 10 (7). While these research groups produce some evidence for both proteins being candidates for EPF, we do not con-

sider that either fulfills sufficient criteria for EPF activity.

Thioredoxin is an ubiquitous protein found in both prokaryotes and eukaryotes. The mammalian protein has a molecular weight of approximately 12 kDa with two cysteine pairs, one in the middle of the protein and the other near the carboxy terminus. The cysteines in the middle of the protein are highly active and confer a redox property on the protein. Thioredoxin has roles in protein folding, in DNA replication, as a redox agent, and in activating glucocorticoid receptors (8). Clarke *et al.* (6) were never able to demonstrate that purified thioredoxin had EPF-like activity in the rosette inhibition test alone, however, they demonstrated that it had EPF-like activity when combined with mouse estrous serum or platelet-activating factor. In our laboratory we were not able to duplicate these results. Further work using site-directed mutagenesis experiments demonstrated that the EPF-like activity of thioredoxin was due to one of the cysteines in the carboxy terminal pair, and not the cysteine pair which confers redox activity to thioredoxin (9). In our laboratory, however, we have shown that thioredoxin (through its reducing power) is able to regulate "late" but not "early" EPF activity (Lash and Legge, in preparation). From these data we propose that thioredoxin is involved in the EPF system as a regulator of EPF activity but there is no evidence to support thioredoxin as EPF.

Recently Cavanagh and Morton (7) used human platelets as a starting material for the purification

of EPF and isolated a protein with homology to rat chaperonin 10 (one of the heat shock proteins) based on the analysis of peptide fragments. It is a mitochondrial protein which acts in conjunction with chaperonin 60 as a seven-membered ring to facilitate the correct folding of proteins (10). To date, chaperonin 10 activity has always been associated with chaperonin 60. Currently there is no evidence that chaperonins function as extracellular proteins, yet EPF activity is detected as a serum factor.

Two factors appear to be crucial when examining these two candidate proteins. First, proof of EPF identity is by using the rosette inhibition test, which is a nonspecific bioassay test whereby the binding of heterologous red blood cells to splenocytes is inhibited by the presence of EPF in serum; it may follow therefore that there could be several proteins capable of exhibiting this activity. And second, both thioredoxin and chaperonin 10 are proteins with known functions associated with protein folding which are being added to a bioassay system containing viable splenocytes. Recent investigations in our laboratory have reinvestigated known proteins with growth factor activity for potential EPF activity using the rosette inhibition test over a wide range of dilutions. Currently we have investigated insulin, insulin-like growth factor-I (IGF-I), insulin-like growth factor-II (IGF-II), prolactin, human placental lactogen, and growth hormone. Of the six proteins investigated, IGF-I was the only protein with EPF activity. In addition, the dilution range associated with the activity was identical to that associated with normal early pregnancy EPF activity. However, both serum and tissue culture samples that had EPF activity did not have detectable levels of IGF-I, although this probably reflected the limitations of the sensitivity of the IGF-I radioimmunoassay, as the EPF activity of IGF-I was associated with picomolar concentrations, whereas the RIA technique was capable of detecting only nanomolar concentrations. Can IGF-I be called EPF, or is this result a vagary of the rosette inhibition test? The second issue relating to the protein folding properties of both of the two proteins previously proposed may well provide conditions for the interaction and synthesis of EPF-like activity by the splenocytes themselves. Currently there are examples of proteins being synthesized as nascent polypeptide chains or as preproteins that become active once they have been folded or have signal peptides removed. Both thioredoxin and chaperonin 10 could have regulatory roles in this process.

Recently we have shown that when mouse estrous ovaries and oviducts are cultured with embryo conditioned media or platelet-activating factor (PAF) and prolactin, then EPF activity is detected in the culture media (11). This system could provide the basis to identify the identity of EPF directly using both protein microchemistry and molecular biology techniques. The use of this approach is currently being investigated in our laboratory.

It is clear, however, that the molecular identity of EPF is still not fully known and that, until it is, our knowledge of the role and mode of action of EPF is severely limited.

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