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NEWS AND VIEWS

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Does "EPF" Have an Identity?

The acronym "EPF" has been around now for more than 20 years as a convenient way to describe the pregnancy-specific capacity of sera to induce increased rosette inhibition titers in the rosette inhibition assay (1). The acronym stands for "early pregnancy factor," although many may have concluded over the past two decades that "enigmatic pregnancy factor" might be just as appropriate, for it has remained as fascinating and frustrating an enigma as ever there was. The fascination stems from the fact that the serum activity described by the use of this acronym is detected in pregnancy but not nonpregnancy sera. Moreover, it can be detected in maternal sera within hours of fertilization, making it the earliest known systemic sign that fertilization has occurred. As its continued presence is dependent on a viable embryo, detection of this biological activity of maternal serum can be used to monitor embryo viability during preimplantation stages of development (2). It is fascinating for the reproductive biologist, or any one else for that matter, to contemplate how such an activity can be expressed systemically in the mother's serum so soon after fertilization. Adding to this fascination is the fact that the capacity to express this activity is not restricted to pregnancy sera. Indeed it is shared with samples derived from a variety of biological sources. Comparable activity expression, or "EPF" as some would have it, can be found in extracts made from regenerating liver, in sera of animals that have recently undergone partial hepatectomy, in platelet extracts, in sera of patients suffering from testicular and gestational tumors, in conditioned media from all sorts of mammalian cells in culture, and even in conditioned media from proliferating yeast cells (3). So in "EPF" we may be contemplating something of fundamental biological significance. But what is "EPF"? This is where the frustration arises, for "EPF" presents as an enigma.

The enigma arises from, and has been sustained by, the following features.

A. Anomalous behavior: The common usage of the acronym has led to the implicit and in some quarters explicit assumption (4) that the biological activity of pregnancy serum (and other biological samples) in the rosette inhibition assay is due to the presence of a simple pregnancy specific protein molecule to be called "EPF." Most of the experimental evidence, however, is inconsistent with the notion that a single factor is responsible; if it is, it must have some very peculiar characteristics (4, 5).
B. Bioassay characteristics: The rosette inhibition assay, while considerably refined over time, has been a difficult and temperamental assay and is still of unknown mechanism and specificity.
C. Complexity and apparent contradiction in characterization: The molecules so far identified as being active in the bioassay, arachidonic acid; leukotrienes B4, C4, D4, and E4;

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and chaperonin 10, and others such as thioredoxin, identified as regulating activity expression in pregnancy sera, are in no way pregnancy specific. All are remarkable for their ubiquitous distribution in biological systems and might well be expected to be components of both pregnancy and nonpregnancy sera.

D. Dialectic exclusion: At times in this field the discussion of the real nature of "EPF" has been anything but dialectical; for example, while even a cursory analysis of the literature indicates complexity, this is often explicitly or implicitly denied.

E. Extremes: The extreme potency of active molecules or active combinations in the bioassay has challenged even the modern techniques of molecular isolation and characterization in bringing unequivocal definition to the phenomenon.

Unfortunately, items A through E have not led to F that could stand for "the factor," but instead it stands for

F. Frustration: An enigma remains to baffle and confuse—even after 20 years there is no clear consensus view of what "EPF" is, or even whether "EPF" exists.

But as we enter the third decade of research in this field, does the enigma that has been "EPF" have to persist? Hopefully not! Not if the main reasons for the confusion and puzzlement can be identified and eliminated. Points A–E above identify the difficult bioassay and the paradigm of "EPF" as a single pregnancy specific protein as the main sources of confusion. The former is being resolved by technical improvements in the bioassay, and by increased stringency in its application, as first recommended back in 1987 by Clarke *et al.* (7), particularly in the appreciation of its dose–response characteristics. The latter requires a change of paradigm, and if this occurs, the enigma disappears. Gratifyingly, there are some signs that this is occurring, as molecular characterization of the molecules and mechanisms that allow pregnancy sera to induce increased rosette inhibition titers in the bioassay are starting to emerge.

Beginning in 1990, a series of studies from this laboratory (for a summary see Ref. 5) has provided consistent evidence for a multifactorial system. Placental preparations, active in the rosette inhibition assay, revealed the presence of a polypeptide of relative molecular mass 12 kDa with associated active moieties of low molecular mass. The protein was identified as thioredoxin. Pure thioredoxin alone does not induce increased rosette inhibition titers. However, when it is applied to spleen cells in combination with, or subsequent to, such cell stimuli as platelet-activating factor (PAF) or normal serum, thioredoxin plays a permissive role allowing for the expression of increase inhibition titers, where none are achieved in its absence. It has been shown to achieve this effect by preventing, or reversing, a refractory state induced in the spleen cell population by these stimuli, allowing probably lipoxygenase-dependent products, also generated in response to these stimuli, or possibly naturally present in the case of sera, to exert their effects in inducing increased rosette inhibition titers (8, 9).

In 1991 our group (8) showed that all sera could stimulate the spleen cells used in the assay to produce potentially active moieties. However, as only sera of pregnant animals caused increased rosette inhibition titers, it has been suggested that pregnancy sera may be distinguished from nonpregnancy sera by the presence of functional forms of thioredoxin or thioredoxin-like molecules, which act to reverse or prevent the refractory state induced by other serum components and, so, allow for increased inhibition titers. Adsorption studies with antithioredoxin antibodies (8) supported these conclusions as to the permissive function of thioredoxin and, also, indicated that there probably is an association in pregnancy sera (but not nonpregnancy sera) between the thioredoxin-like proteins and some active moieties of low molecular mass.

This multifactorial model of the capacity of pregnancy serum to express activity in the bioassay, rather than needing to "be reinterpreted" (10), has now been substantiated by recent studies (6, 11) which defined in principle the basic set of components required, and which explained many of the previously enigmatic characteristics of "EPF" as simply properties of the system of components responsible for activity expression.

Cavanagh and Morton (4) have recently suggested that chaperonin 10 is "EPF." This was based on the discovery that isolated chaperonin 10 appears to be active in the bioassay and that it can be isolated from sources that express "EPF" activity. Also, some evidence was reported that removal of chaperonin 10 by affinity agents removes the capacity of the source material to dis-

play activity in the assay, indicating (in a minimalist interpretation) a role for chaperonin 10 in the processes that lead to activity expression. While Cavanagh and Morton imply that this molecule is solely responsible, it is interesting to note even here some shift in paradigm with the suggestion that it ("EPF"/chaperonin 10) may be "the molecular entity initiating response in the EPF bioassay." This could be so, but given the evidence reviewed above, it is unlikely that it is solely responsible for the bioactivity of pregnancy serum. Adsorption of pregnancy serum with antithioredoxin antibodies also removes the capacity to display activity. Does this mean that thioredoxin is also "EPF"? No; it has never been suggested so. Rather, thioredoxin has been shown to be a permissive factor in the system of components that ensures activity expression (11). Chaperonin 10 may be another of the required components.

While our forebears of the Victorian era would be shocked to learn that a chaperone was involved in setting up pregnancies, this may be so. However, as with many of the social attitudes of that era which were too simplistic by present-day standards, the acronym "EPF" may also become an anachronism. Future studies utilizing the molecular leads and models reviewed above should not only resolve how pregnancy serum induces activity in the rosette inhibition assay, but also reveal things of greater physiological fascination—the potential extracellular roles of thioredoxin (12) and possibly chaperonin 10 (4) in essential reproductive processes.

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