An Update on the Identity of Early Pregnancy Factor and Its Role in Early Pregnancy¹

The immunologic tolerance of mother and fetus is an intriguing example of a successful allograft in nature. Considerable research has been done in the past few decades in an attempt to understand this phenomenon. Altered maternal immune reactivity by a pregnancy-related factor(s) having immunomodulating activity is one possibility currently favored.

Early pregnancy factor (EPF) is described as immunosuppressive activity detected in pregnancy sera by means of the rosette inhibition assay (1). The activity of EPF has been detected in the sera of mice, humans, and sheep from 6 to 24 hr after conceptive mating and persists for at least two thirds of the gestational period (1–3). Isolated EPF from human embryo growth media of in vitro fertilized ova was shown (4) to have suppressive activity on concanavalin A (Con A-stimulated lymphocyte proliferation assays). The presence of EPF and immunosuppressive activity in the growth media of cleaved ova and not in the media from unfertilized ova (4–6) indicates that this factor is indeed a product of the early developing embryo. The early pregnancy factor that was detected by its suppressive property on Con A-stimulated lymphocyte proliferation assay was named the "embryo-associated immunosuppressor factor (EASF)" and was demonstrated to be secreted by human preembryo (7–9) (in vitro fertilized embryo; referred to as preimplantation EASF) and to be present in pregnancy sera (7, 10) (postimplantation EASF). Whether EPF (immunosuppressive activity of the rosette inhibition assay) and EASF (immunosuppressive activity in the Con A-stimulated lymphocyte proliferation assay) are related is not known.

The physicochemical properties of EPF are ill defined; molecular weights may range from 20 to 200 kDa (11). Some variation has been attributed to an association of EPF with carrier proteins (12). Studies in sheep (12) and mice (13) indicate that EPF consists of two components, termed EPF-A

and EPF-B. Serum concentrations of the various EPF forms and their components vary from species to species and are dependent on gestational age (12, 14). Although some attempts have been made to purify and characterize EPF from human sera, the biochemical nature of human EPF is still unclear. EASF purified from human embryo growth media and pregnancy sera were identified as 14, 24, 30, and 80 kDa (7, 9). Further studies done on these EASF fractions from the two sources demonstrate that they suppress both T- and B-cell proliferation, probably by binding to specific receptors expressed by lymphocytes after mitogenic stimulation (10). Although the exact mechanism(s) of EASF-induced immunosuppression is not clear, the data offer presumptive evidence that EASF affects T-cell and B-cell functions directly. This suggests an active process by which the fetus might abrogate proliferation of maternal lymphoid cells, thus preventing the mother from responding against the fetus.

EASF activity in embryo growth media (second 24-hr embryo culture media) has been correlated with successful pregnancy (6, 8, 15) in patients undergoing in vitro fertilization and embryo transfer (IVF-ET). In maternal serum, a number of pregnancy-associated factors have been reported to have suppressive effects on mitogen-induced lymphocyte proliferation, such as pregnancy-associated glycoproteins (16), α -fetoprotein (17), progesterone (18), and human chorionic gonadotropin (hCG) (19). Preimplantation embryos have been shown to secrete several hormones and factors, such as platelet activating factor (20), SP1 (21), prostaglandin E₂ (PGE-2) (22), and a high molecular weight glycoprotein (23) that inhibit lectin-induced lymphocyte proliferation. It is possible that early pregnancy factors (EPF/EASF) may act in conjunction with one or more of the above-mentioned hormones and/or factors to maintain a successful pregnancy. This hypothesis has been supported by several findings in which (i) an association of EASF activity in embryo growth media, estradiol (E₂) levels in IVF patients' serum, and success of pregnancy (24) were demonstrated in IVF-

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ET patients; (ii) levels of serum EASF, E_2 , and follicle stimulating hormone (FSH) were suggested to act synergistically in the development of ovarian follicles in patients undergoing artificial insemination programs (25); and (iii) serum EASF in association with serum E_2 levels was suggested to play a role in the preembryonic cell division and success of pregnancy (26) in IVF-ET patients.

Murine monoclonal antibodies (Mabs) were produced against preimplantation EASF (EASF isolated from human in vitro fertilized embryo growth media). These EASF-specific Mabs were able to recognize and neutralize biologically active immunosuppressive components in embryo growth media (27). Recently these Mabs were used to immunohistochemically localize EASF in human placental tissues (28). One of the EASF-specific Mabs (H5D12) showed intense immunostaining in both villous syncytiotrophoblast and cytotrophoblast cells of first- and second-trimester placental tissue; the intensity of which was weaker in the third-trimester placental tissues. The strong reactivity of the Mab H5D12 toward first-trimester trophoblastic cells (rapidly dividing) prompted the examination of choriocarcinoma cell lines which are established from malignant placental trophoblasts for the presence of EASF (29). Interestingly, the Mab H5D12 also stained the BeWo choriocarcinoma cells but not the placental cell line (3A-SubE) and the control cell lines [hepatoma (HEP-2) and leukemia (K562)]. The metabolic labeling and EASF bioassay studies demonstrate that the BeWo cell line-secreted factor has a molecular weight of 24 kDa and possesses immunosuppressive activity (29).

In addition, EASF secreted by in vitro fertilized human ova and placental villi has been identified by the Mab H5D12 as a 37-kDa molecule that possesses immunosuppressive activity (28). One possible explanation for the different molecular weight factors identified by Mab H5D12 is that these represent isoforms of the same protein, but this idea awaits further study. It is possible also that the molecular form (37-kDa molecule with a different three-dimensional structure) of EASF secreted by malignant placental trophoblasts is different from that secreted by human preimplantation embryos and first-trimester placental villi. The presence of this factor in placental tissues and cells of trophoblastic origin demonstrates the probable site of production of EASF in a placenta and further implicates this factor as one of the key regulators of reproductive functions.

The mechanism by which these suppressor factors may result in successful pregnancy is not clear. However, they might have several implications in vivo. For instance, it is evident from several studies (30) that traffic of lymphocytes normally occurs through the placenta. Therefore, the suppressor factors may prevent the proliferation of maternal T and B lymphocytes that crossed the placenta into the fetal circulation. Also, maternal lymphocytes coming in contact with the fetal, antigenically alien, trophoblastic cell layer covering the villi might be prevented from responding by proliferation and a graft-versus-host reaction. Although the in vitro studies show the immunomodulatory effect of EASF, the physiological significance of EASF depends on whether it is present at sufficient concentrations at sites where immune suppression is desirable and whether it is immunosuppressive under in vivo conditions. If placental tissue does secrete an immunosuppressive agent(s) that inhibits fetal rejection by the mother, new ways to manipulate the immune system for possible therapeutic ends may be achieved. Abnormal EASF activity associated with certain reproductive diseases may be identified. The knowledge of the association of early pregnancy factors (EPF, EASE, and/or others) with pregnancy outcome will help us understand the clinical problems of unexplained infertility and will offer new insight into the facilitating phenomenon of maternal-fetal coexistence.

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