The Viruses That Make Us:

A Role For Endogenous Retrovirus In The Evolution Of Placental Species

by Luis P. Villarreal

Chromosome evolution, higher order and parasitic elements. With the accumulation of genomic sequence data, certain unexplained patterns of genome evolution have begun to emerge. One striking observation is the general tendency of genomes of higher organisms to evolve an ever decreasing gene density with higher order. For example, E. Coli has a gene density of about 2 Kb per gene, Drosophila 4 Kb per gene and mammalian about 30 Kb per gene. Much of the decreased density is due to the increase in the accumulation of non-coding or 'parasitic DNA' elements, such as type one and two transposons. Current evolutionary theory does not adequately account for this observation (81). In addition mammals appear to have retained the presence of at least some copies of non-defective 'genomic retroviruses', such as intercysternal A-type particles (IAP's) or endogenous retroviruses (ERVs), (51,85). It is currently difficult to account for the selective pressure that retains these genomic viruses, since they often lack similarity to existing free autonomous retroviruses. It is widely accepted that viral agents act a negative selecting force on their host. However, viral agents have very high mutation and adaption rates. This character led Salvador Luria to speculate early on that perhaps viruses contribute to host evolution (52).

There is now sufficient evidence to suggest that horizontally transmitted agents and gene sets allow the rapid adaption of various living systems, including bacteria, yeast, drosophila and hymenoptera. 'Pathogenic islands' are contiguous regions of DNA that contain gene sets in bacteria that appear to be horizontally acquired and can exist as either prophage, episomes or genomic sequences (21). These pathogenic islands appear to account for much of the rapid adaptability in bacteria. Transposons of Drosophila appear to require horizontal transmission in order to be maintained during evolution and appear to have been the underlying mechanism of hybrid dysgenesis (10). The parasitioid wasp species (hymenoptera) maintain genomic polydnaviruses in most species which are highly produced into non-replicating viral forms

during egg development and subsequently suppress host larval immunity making them essential for egg survival (47,74). Thus horizontally transmitted genetic elements are common in the genomes of all species.

The mammalian chromosome presents an especially interesting case of accumulation of 'parasitic' DNA. All placental species have unique LINE elements present at very high abundance as well as other related and even more abundant elements, such as the SINES or primate specific alu elements (see (70) for references). Yet there appears to be no common progenitor to these elements. All these elements appear to be products of reverse transcription of cellular RNA's however, there is no explanation for the conservation to RT activity in mammals.

Although endogenous retroviruses are found in most organisms prior to mammalian radiation, the levels of these genomic agents is relatively low in non-mammals and the nature of retroposons seems distinct form that in mammalian. Mammalian LINES, for example lack a precise 5' end, have no poly-A 3' end, and lack RT coding regions that are characteristic of all LINE elements as opposed to avian or other retroposon elements of vertebrates that do not have these features. Why are mammalian (eutherian) chromosomes especially so full of these RT derived agents? What selects for their generation or retention?

A genomic retrovirus: essential for placentals? In a proposal published in 1997, I raised the issue of endogenous retrovirus and proposed that these viruses are essential to the biology of Eutherians. Viviparous mammals confront an immunological dilemma in that mammals which have highly adaptive immune systems fail to recognize their own allogenic embryos (58). The relationship of mammalian mother to her fetus resembles that of a parasite and host in that the fetus 'parasite' must be able to suppress the immune response of the 'host' mother in order to survive. As viviparous mammals are also noteworthy for having genomes that are highly infected with endogenous retroviruses and as retroviruses are generally immunosuppressive, the possible participation of endogenous retroviruses in the immunosuppression by the embryo was then considered. In addition, it was considered if such endogenous viruses might be more broadly involved in

the evolution of their host and the resulting host genome that now appear to have many derivatives (such retrotransposons and as LINE elements) of such genomic viruses. This grant application seeks support to do an experimental evaluation in a mouse model of the proposed involvement of endogenous retroviruses in the immunologically escape by the embryo in the mother. I argue that endogenous retrovirus is hence essential for the biology of non-egg laying placental mammals. This study could provide evidence of the biological function of endogenous retroviruses and also address the broader issues concerning the possible contribution of genomic virus to host genome evolution.

The dilemma of viviparous mammals and their allogeneic embryos.

This mammalian dilemma was clearly stated by Medawar in the early 1950's, see (31,50). Since then, this dilemma has remained one of the most vexing and persisting problems in immunology. An array of models have since been proposed attempting to explain this situation. These include a limited embryonic expression and presentation of MHC class I or class II antigen ((88) or expression of alternative MHC, HLA I-G, (40), or a high hydrocortisone hormonal suppression of immunity, and more recently the possible role of Fas L embryonic expression in ablating T-cell recognition of the embryo (66). All of these models, though with some support, have significant problems. Inflammatory reactions, which appear to be involved in embryo rejection (see below) would not be checked by low MHC expression. Lowered MHC I expression would also be expected to elicit a natural killer cell response, which appears to be important in embryo implantation (41), although the human embryo specific alternative MHC gene, HLA-IG, could substitute for MHC I to negatively regulate NK activity. Up regulated expression of Class I MHC by interferon does not allow CTL killing of trophoblasts suggesting that trophoblast actively inhibit CTL killing (for references, see (26)). Also, humans with deficient beta-2 microglobulin do not express HLA-IG yet the fetus comes to term indicating HLA-IG is not essential for implantation (James Cross, personal communication). In addition, other species, such as mouse have no analogue of the HLA-IG antigen, which suggest this antigen cannot be a general solution to the immunological dilemma of viviparous species. Fas null mice, although displaying defects in peripheral clonal immune selection, allow implantation

of embryos (1). General immune suppression, such as hydrocortisone cannot explain the relatively normal immune response in pregnant mothers to many agents or elevated level of T_H2 reactive cells (which are important for mucosal macroparasite elimination) seen in pregnant woman, see T. Mossman, (49). Also, the glucocorticoid effect may be mediated via the p15E-like gene of endogenous retrovirus (20). In addition, it is interesting to note that autoimmunity, such as rheumatoid arthritis can often abate during pregnancy suggesting an altered immunity that appears not mediated via hormones (Fackelman, Science News 144:260). Most effective immune reactions appear to be of a rather local nature. Therefore local suppression seems a likely way to regulate embryo immune recognition. Although T_H1 reactivity in pregnancy is weak, the T_H2 response, which is important for inflammatory like reactions, is not decreased and is possibly enhanced (T. Mossman). Neither the MHC model, nor the Fas-Fas ligand model can account to the failure to initiate an inflammatory reaction or NK activity against the embryo (activated NK cells can reject xenografts (41))

The Role of Mucosal Uterine Macrophages or NK cells

Embryo implantation occurs in the mucosal epithelial tissues of the uterus. Like most mucosal surfaces, the uterus has a high abundance of macrophages (37,70) and NK cells (41). Once activated, these cells should respond vigorously to parasites or allogeneic tissues and reject xenografts. The regulation of these cells and their subsequent inflammatory reaction and induction of the adaptive immune response involves IL-1 beta, IL-6, TGF beta-1, TNF-alpha, CSF-1 (26,88). The uterus appears to present an immunologically tolerant site as grafts into the uterus of pregnant rats have prolonged survival relative to other locations, see (5) for review. Macrophages are central to the initiation of innate and subsequent adaptive immune responses (18). Although most macrophages can act as immunostimulatory cells, evidence suggest that uterine macrophages can make immunosuppressive molecules. For example, despite MHC II display, uterine macrophages don't present antigens to T-cells (44).

Other results suggest that uterine macrophages can contribute to embryo loss. Preterm mouse delivery is associated with high levels of macrophage derived

IL-1-beta, IL-6, TNF- α . High rates of early embryo loss can be associated with the specific mouse strains that are mated in that low rates of embryo loss can sometimes be seen with inbred crossings, whereas some outbred crossings can show higher embryo loss rates. For example, crosses between CBA/J X DBA/2 are prone to early embryo loss relative to inbred crosses which is enhanced by IFN induction (27). This breeding associated embryo loss is also linked with inflammation and iNO production by local decidual macrophages (27) as inhibition of macrophage iNO enhanced litter size. Macrophage iNO inactivates nearby macrophages and mediates immunosuppression in inflammation via bystander lymphocyte autocytotoxicity, suggesting a way to elicit immunosuppression.

The Importance of Trophoblast

Role in implantation. In the implanting embryo, trophoblasts are the first cells of the egg to differentiate. Following the loss of the zona pellucida shell, trophoblast differentiate into cytotrophoblast the finally into the fused syncytiotrophoblast that forms the cell layer that directly contacts the uterus and the mothers blood system. These trophoblasts are considered a part of uterine macrophage-cytokine network (26,88). Trophoblast resemble macrophages in many of the genes that they express. Uterine macrophage produced IL-1 which may play critical role during implantation (28). Trophoblasts protect inner cell mass from macrophage destruction (69). Trophoblast can be transplanted across mouse strain barriers without being rejected suggesting they have immunosuppressive activities (38). Also, trophoblasts have a very unique pattern of gene expression in that expression is restricted to paternal (androgenic) genes while inner cell mass express maternal genes (79). This is in stark contrast to other somatic tissues where mosaic expression is observed. With trophoblast gene expression being androgenic (79,80), it seems curious that X chromosome inactivation is also paternal in trophoblast, see Renfree (61) for references. It is interesting therefore to note that female mice are less able to kill tumors bearing paternal antigens then tumors bearing maternal antigens (T. Mossman, personal communication). Trophoblasts are intriguing in an evolutionary sense as well. Other non-viviparous mammals (marsupials, monotremes) completely lack the trophoblast-syncytiotrophoblast layer, see (59) for review. Unlike viviparous mammals, marsupial gestation is short (averaging several to 12 days), their eggs are yolk-filled resembling those of reptiles and marsupial eggs are surrounded by a maternal derived shell membrane which once lost allows only

minimal maternal-fetal contact for a period of only several days. Most of marsupial egg incubation is outside of mothers body and birth is associated with local inflammatory events. Marsupials also lack hormonal control of uterus or other tissues (61). Given that the trophectoderm is the first mammalian egg cell type to differentiate and the relatively recent evolutionary development of this layer in mammals, early embryos of the viviparous mammal do not seem to recapitulate evolutionary history with respect to this first cell type. Thus the trophoblastic cells appear to be centrally involved in implantation and embryo immunomodulation.

Trophoblast produced ERV's. Another rather unique feature of syncytiotrophoblasts is in their ability to produce a high quantity of endogenous retroviruses , see (85). This also appears to be a general characteristic of all placental mammals. The production of endogenous retroviruses in early mammalian embryos is a long standing and often repeated observation. Multiple detections of particles in normal human embryonic cells, especially basal surface of human placental syncytiotrophoblast tissue have been frequently reported as have similar particle production in old and new world primates placentas (for early review see (84)). Normal human placentas have measurable RT activity (56) and appear to express HERV *env* gene (45). Primary trophoblasts of rhesus monkeys also produce ERV's (77). Furthermore, the levels of mouse virus particle production can be as high as 10^5 per cell (60), which exceeds by far the capacity of most permissive cell culture systems for retrovirus production. In addition, these endogenous retrovirus particles are frequently made following induction in testicular teratocarcinoma which constitute a HERV (Human Endogenous Retrovirus) group, similar to C-type particle (85). In addition, antibody studies have established that CTL reactive to ERV proteins can be found in most pregnant woman as can immuno-precipitation reactivity to p28, p15 and p15E (for references see (85), p. 86-87). Interestingly, RD114 cross-reactive antibodies were significantly correlated with complications during pregnancy and with prior abortions and stillbirths (78). In humans, these trophectoderm expressed HERV's represent two large diverse multi copy families HERV-R and HERV-K., the latter is capable of expressing the *env* and p15E gene products in vaccinia vectors (83). Thus, endogenous retrovirus are mainly isolated from reproductive embryonic tissues but to a lesser extent from circulating lymphocytes or monocytes of some mouse strains (42). These viruses are highly suppressed in most somatic tissues probably due to DNA methylation, (see below). However, these viruses do not seem transmissible in usual sense of leading to productive infections.

Nondefective endogenous retroviruses are conserved and expressed in trophoblast

HERVs constitute about 0.6% of the human genome and appear more related to rodent viruses than any known human viruses. The great majority of these endogenous viruses are defective and deleted of various gene products, especially the *env* gene but also *gag/pol*. For an early review of the human endogenous retroviruses see (46). Initially, it was felt that there all copies of HERV's in the genome were defective, but it subsequently became clear that highly conserved non-defective copies also exist at low levels (see Urnovitz (85) table 6 , p.93 for refs.). For example, the HERV-K sequence of the human teratocarcinoma derived virus type (HTDV), is reported to be able to make retrovirus like particle and can express *gag*, *pol* and *env* genes via vectors (83). Also, ERV 3 can express *env* gene in embryonic placental tissues (45). Such reports may now explain the numerous early observations of being able to find viral particles in human tissues (13), (see (33) for early references). Although some HERV's are expressed in mammary tumors, the feline RD114, ERV-3, and HERV K10+ are all expressed in placental tissues. What then is the significance of nondefective ERVs and why is expression so common in embryos?

There has developed a confusing system of nomenclature and corresponding phylogenetics of ERVs due to multiple names for similar viral sequences. In addition, sequences from several ERV's appear to be made up of mosaic elements such that different relationship will be apparent when different parts (e.g. *gag*/*pol* vs *env*) are analyzed as seen with HERV-K10+, which can add to confusion (85). A relatively clear system of nomenclature has been presented by Urnovitz and Murphy (85). They propose HERV's can be classified according to established non-defective endogenous viruses. For example, both the ERV-1 (with a deleted env region) and the single copy ERV-3 (which can placentally express an intact *env* gene) are also called HERV-R (45) can be classified as ERV-3 derivatives. Accordingly, the defective HERV-K10 with deleted *env*, or the non-defective full length HERV-K10+ and the HERV K(C4), are thus related to HERV-K10+. In addition, RTLV-H, in which most copies are *pol* defective but is also expressed in embryonic tissues and also has an *env* gene (32), is present as a

low copy nondefective copy; RTLV-Hp. Interestingly, this RTLV-Hp sequence appears to have been conserved phylogenetically (via neutral codon substitutions) and implies that it belongs to a functional and selected subclass of highly retained ERV's (89). This classification method allows clearer identification of highly conserved and intact ERVs. What could an ERV function be for the host cell? I (68) and Venables et al. in the Boyd group (8,86) have proposed that some of these HERV's may function during embryo implantation to help prevent immune recognition by the mother's immune system.

Immunological activity of ERV (IAP) genes

Most retroviruses appear to be generally immunosuppressive of the host immune system (for review see (25)). The immunosuppressive nature of retroviruses was first investigated in detail with feline leukemia virus of domestic cats (FeLV) and led to the identification of the CKS-17 hydrophobic transmembrane domain of the *env* gene as an important immune modulator. This domain is present in the highly conserved p15E peptide which maintains the immunosuppressive character, for review see (30). A main effect of p15E is to inhibit T-cells via cytokine (TNF and IFN) mediated processes (29) and can be elicited by synthetic or recombinant p15E (65,67). p15E also inhibits mononuclear phagocyte chemotaxis (85). Thus the *env* gene is a primary candidate of an ERV gene product that could modulate the mother's immune recognition, which fits well with its proposed role in syncytiotrophoblast expression. In addition, the ERV *gag* gene product may also be immuno-modulatory. The p70 (*gag*) of mouse IAP has been cloned and expressed and shown to be identical to IgE binding factor (IgE-BF) which is a regulator of B-cell ability to produce IgH (43,54). More recently, it has been reported that endogenous *gag* is Fv-1, an-Herv.L like endogenous virus which confers resistance to MLV tumors (7). Although some researchers disagree with the immunomodulatory role of p15E, an immune suppressing activity in culture assays has been clearly established. These supporting results seem sufficiently clear to warrant a serious investigation that both the *env* and *gag* gene products of ERV's may modulate immunity.

ERV's and placental macrophages

If non-defective ERV gene (*env*) products are indeed immuno-modulatory, we can now offer explanations for various other observations. For one, *env* expression should be highly selected for in the early embryo (hence the conserved single intact copy), but strongly counter selected for expression in ectopic sites which would render these genes inappropriately immunosuppressive. Therefore most transposed copies of ERV's would be expected to be under selection to lose the *env* gene, as is observed. Also, ERV expression is somatic tissue is generally highly repressed, also as expected from this model. In addition, it can be expected that the main target of ERV action would be the local immune cells of the uterus. A likely cell type to affect would be the uterine macrophages. Given the central role of innate immune modulators (18) and macrophages (2) in the induction of the acquired immune response, uterine macrophages and the cytokines they effect seems a likely candidate to target for embryo immune regulation. However, there is no evidence that ERV's are transmitted in a productive manner. We therefore might expect the trophectoderm derived ERV's act more like a replication defective recombinant retrovirus that is able to effect locally exposed cells, but not replicate and transmit to other cells (see (87). This would mean that these ERV's are essentially local acting agents. Thus a central unanswered question is what effect IAP producing trophoblasts have on nearby macrophages, especially with respect to a macrophage's role in innate and acquired immune function. Of some relevance to this issue are reports glucocorticoid mediates increased Mtv *env* (p15E) expression in P388D1 macrophage and T-like mouse line (20). Such cell systems could be used experimentally to examine possible role of *env* in immune modulation.

One seemingly contradictory observation concerning the above proposal is that normal embryo development appears to occur in the presence of inhibitors of reverse transcriptase, AZT, such as in AZT treated HIV infected mothers which generally produce normal offspring. If the embryo produced ERV's are needed for immune modulation, it seems likely that embryo's would be immunologically rejected if RT inhibitors prevent the production of ERV's. However, early embryo development is severely affected by AZT, see (82). AZT will efficiently inhibit normal embryo's at post fertilization but preimplantation stages. AZT is toxic to early embryos at before blastocyst stage however, but it is not toxic at post blastocyst implantation stage (82). The possibility that embryos were being rejected by the mother's immune system was not examined in these studies. An additional consideration concerning the possible use of RT inhibitors is that because the ERV's are

being produced in the trophectoderm from genomic copies of virus, RT inhibitors are not expected to inhibit trophectoderm produced ERV's as viral genomes are already integrated as DNA (88). Support for this comes from HIV studies showing that AZT did not inhibit HIV gene expression in infected placental trophoblasts. However, it might be predicted that local immune cells, such as uterine monocytes or macrophages, might not be properly 'reprogrammed' to immune nonrecognition by ERV's infection as the integration step in these cells would be inhibited. Once these macrophages were reprogrammed by ERV infection, their 'anergic' state could persist rendering them resistant to further RT inhibitors as long as the cells live, which is seldom known for these cell types. Clearly, this issue should be examined experimentally.

IAPs and cancer

IAP expression, although normally highly repressed, is often observed in various tumor tissues (14,15,90). If these ERVs are a normal host system of immune modulation as I have proposed, it could be expected that tumors would select for the expression of immuno-modulatory ERV or ERV gene products (such as p15E) in order to avoid immuno-surveillance. Early reports presented evidence that p15E is made in many human breast cancers (73). This suggest that tumor cells might also be used as an experimental system in which to examine this issue. In some tumors, there appears to be interesting converse links between IAP expression and tumor recognition. BL6 melanoma normally make high levels of IAP and do not express H-2kL^{d} · IAP production can affect IgE production and is conversely is lost when MHC-I H-2kL^{d} ,and H-2kL^{b} but not H-2D^{d} H-2L^{d} is transfected into BL6 cells (48). Also, P15E-like proteins in serum, urine and tumor effusions of cancer patients suppress immune responses that can be reversed by anti-p15E antibody (71,73).

ERV (IAP) genetics and implications for the functional subsets.

Because human and mouse ERV's are present at about 900 copies per haploid genome, a genetic analysis would appear to present a daunting if not impossible task. For example, gene knockout experiments in mice, which have been so valuable at elucidating gene function, would seem not possible in the context of IAPs. However, intact *env* genes are sometimes present at much lower levels, and in some cases as single copies (ERV-3). It seems likely that this limited subset is the functional set that might be important. ERV-3 seems like a very good candidate that could provide immunosuppressive barrier between human mother and fetus as it has highly expressed *env* in syncytiotrophoblasts, expresses antigens that react to antibodies specific to the transmembrane domain (p15E-like), and is present as a complete, single copy sequence on chromosome 7, (Larsson, '97 NEED THIS) (86). Other good candidate human ERV's are the HERV-K(C4) and HERV-K which are also highly expressed in the placenta. Interestingly, Y human chromosome has lots (20) of different ERV's related to ERV3 (Kjellman, Sjogren, Widegren, '95, NEED THIS) which may code for potential HY antigens.

However, what is really needed for experimental analysis is the mouse homologue to the human ERV-3. One possible functional homologue is the IAPE virus which like ERV-3, has an intact *env* sequence (62). In addition, this IAP env sequence appears to be expressed as a protein in NH15-CA2 cell lines suggesting a functional gene (62). The IAPE sequences, however, are complicated by the existence of about 200 copies/cell in *mus musculus* (63). But the IAPE-A locus seems complete and intact relative to the other IAPE's which lack gag or pol sequences and IAPE-A is present at lower levels. IAPE's are found in all lab strains (mostly *Mus musculus domesticus* derived) in variable and genetically unique levels that identify the strain (12), suggesting an unexplained link of inbreeding to IAPE variation. Some outbred strains, such as CE/J, had much lower levels of IAPE sequences, but maintain IAPE-A (75). These CE/J mice might offer a simpler genetic system to investigate the possible function of IAPs. Yet, mouse strains do not seem to vary much with respect to the very massive RNA levels (10^5 copies per cell) of early embryo expressed IAP (60). As IAPE-A is complete and it also codes for intact env sequence, this seems like a logical but untested candidate for possible trophectoderm expression. IAPE-Y is an IAPE-A so named because it has amplified on Y-chromosome. However, the Y-amplified head to tail copies are not found in all *musculus* species indicating that this amplification appears to be a recent evolutionary change (19). The repetitive head to tail Y-copies of IAPE are limited to only male *Mus musculus domesticus* and the asian *Mus musculus molossinus* and *M. Musculus castaneus*. The more distant Spanish *Mus spretus* lacked the repetitive copies on the Y chromosome, but

conserved IAPE-A. MuRVY is genetically associated but distinct from IAPE, is also on Y and could represent a second class of trophectoderm expressed IAPs (17,19). Y condensation in most tissue (except testes Sertoli cells) probably limits expression of these IAP-Ys. However, IAPE-A expression, (also related to Hamster H-18 IAP (3)), although usually highly repressed in most tissues, may at times be expressed in some somatic (thymus) tissues of some mouse strains (42). Phylogenetic studies suggest that this *env* gene was under functional constrains not to evolve quickly, although the defective copies are evolving very rapidly. Thus IAPE-A seems like a good candidate for an ERV *env* gene involved in mouse embryo implantation. However, it has not previously been established that this *env* sequence is expressed in trophoblasts (see preliminary results below).

The possible relevance of ES and EC cells. It has long been established that some testicular derived teratocarcinoma cells can differentiate from embryonal stem cells into several cell types (76). Of particular interest is the capacity of some EC lines to differentiate into trophectoderm. Treatment with 10^{-3} M retinoic acid will differentiate some of these cells into parietal trophectoderm-like cells which will eventually develop structures resembling a 3.5 day blastocyst. Thus this tissue resembles the extra-embryonic trophectoderm that is the proposed source of immunosuppressive ERV's. Along these lines, it has also long been established that differentiated (but not undifferentiated) mouse EC cells induces high levels of two distinct populations of IAPs (36). Thus at least by this parameter, EC cells my accurately model trophectoderm gene specific control. Other reports show IAP production in differentiated EC cells can be significantly reduced without affecting the ability of these cells to differentiate into trophectoderm. F9-EC cells containing integrated SV40 sequences (F912-1 cells), resulted in IAP production that was significantly reduced after differentiation. In these cells, it appears that IAP expression is tightly linked to DNA methylation and that SV40 has affected methylation without affecting cell specific expression (34). EC cell differentiation has been well characterized and many expressed sequence tags have been catalogued (57). It should therefore be possible to accurately determine if the EC differentiation program is otherwise affected by SV40 T-Ag or other regulatory proteins.

Historically, EC cells were also used to study cell specific replication by polyomavirus. This led to the development of enhancer variants of polyomavirus that had increased capacity to replicate in undifferentiated EC

cells. Using the enhancer/origin from Py (PyF101), Gassmann et al. with P. Berg constructed a Py T-Ag expressing plasmid (PMGD20neo) that allowed for episomal selection in ES cells (9,24). This plasmid had the interesting capacity to be stably maintained as an episome in ES cells without integration. Some of the resulting ES cell lines could then be used to make mosaic mice that also maintained the Py plasmid. Thus it seems clear that the presence of Py T-Ag expressing DNA was not detrimental to the development of most normal mouse tissues. This plasmid could offer a very useful experimental tool for the genetic analysis of ES and EC cell function (see below).

Another interesting use of EC and ES cells concerns their ability to grow into masses (tumor-like) in the more immunologically privileged site of the brain. Both ES and EC cells can be differentiated into trophectoderm containing embryoid bodies. These embryos will generally grow in various transplanted sites only with immunosuppression. However, following brain implantation of embryoid bodies, ES cells will grow rapidly into large masses (91). Implantation of 2-4 cell embryos, which lack trophectoderm, however, do not grow. It seems possible that the capacity of the embryoid tissues to grow in the brain might be related to the presence of trophectoderm. If so, this might offer another useful experimental system for the analysis of a more limited immuno-modulatory function of trophectoderm and ERVs.

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