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No cancer in cancers: Evolutionary trade-off between successful viviparity and tumor escape from the adaptive immune system

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Summary Some invertebrate species including the king crab and king squid enjoy relatively long lives of up to 20 years. Nevertheless, there are few reports of malignancies among invertebrate animals while there are many such reports in lower vertebrates such as in fishes, amphibians, and reptiles.

Viviparity is characteristic of most mammalian species, although it has been observed in both vertebrate and non-vertebrate species.

As adaptive immune responses evolved among the cartilaginous fishes by virtue of gene duplication, all viviparous vertebrates cope with specific immune responses to reject the fetal allograft.

The escape mechanisms employed by both human and animal malignancies share common properties, which are also employed by feto-placental units, such as the expression of non-classical major histocompatibility antigens (HLA-E, HLA-F, and HLA-G in humans), accumulation of regulatory T cells, Th2-directed immune responses, Fas/FasL- and/or PD-1/PD-L1-induced apoptosis, and the expression of indoleamine 2, 3 dioxygenase which starves the local tryptophan supply that is indispensable for an effective cytotoxic T cells response.

In humans, a single cancer cell requires 1–10 years to develop into a clinically remarkable tumor. For cancer cells, the genes encoding the immunoregulatory mechanisms employed by feto-placental units could be of value for escaping the host immune system.

Taken together, these observations support the author's hypothesis that the evolution of viviparity resulted in an evolutionary trade-off that may have increased susceptibility to malignancies.

Introduction

Viviparity is defined as the retention of developing embryos within the oviducts throughout development, a capacity which evolved more than 100 in different types of animals [1]. From an analysis of the fossil records and existing squamous reptiles, it was proposed that the evolutionary transition from oviparity to viviparity could occur quite rapidly, and that placentation evolved concurrently with viviparity [2].

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The animal kingdom is divided into two main categories: vertebrates and invertebrates. Invertebrates are considered to be all those animals that are not in the five main groups of vertebrates (Mammalia, Aves, Reptilia, Amphibia, and three classes of fish).

Some species of cartilaginous fish, amphibians, reptiles, and most mammals, except for platyps and echidna, are viviparous. Since jawed vertebrates have an adaptive immune system, it is crucial that these animals be able to protect their concepti from an alloimmune response.

Matsunaga proposed the hypothesis that the first adaptive immune system evolved as a gastrointestinal defense against microbial invasion as a result of increasing traumatization by injury and infection due to jaw development in host fish, which allowed them to capture and swallow foods (viz., other fish) [3]. He supported his hypothesis with the histological finding that the gut-associated lymphoid tissue (GALT) system is not developed in the hagfish or lamprey, which capture their food by a gentle sucking action (Fig. 1).

Neoplasms in lower animals

Neoplasias in invertebrates and evolutionary conservation of innate immune system

Proliferative lesions have long been recognized in invertebrate animals as well as in vertebrate animals. However, most of the reports on invertebrate neoplasms have focused on a small number of species including the fruit-flies (*Drosophia melanogaster*), cockroaches (*Periplanta* spp. and *Leucophaea maderae*), locusts (*Locustus migratoria*), beetles (*Melontha melontha*), and stick insects (*Carausius monosus*) [4]. Though the population and number of species of invertebrate animals are much greater than those of vertebrates, it is rare for biologists, fishermen, or cooks to encounter natural neoplasms in invertebrate animals, even if working regularly with arthropods and mollusks.

However, innate immune responses against malignancies have been reported in lower animals. Suzuki and Cooper reported on the spontaneous cytotoxicity of earthworm leucocytes (coelomo-

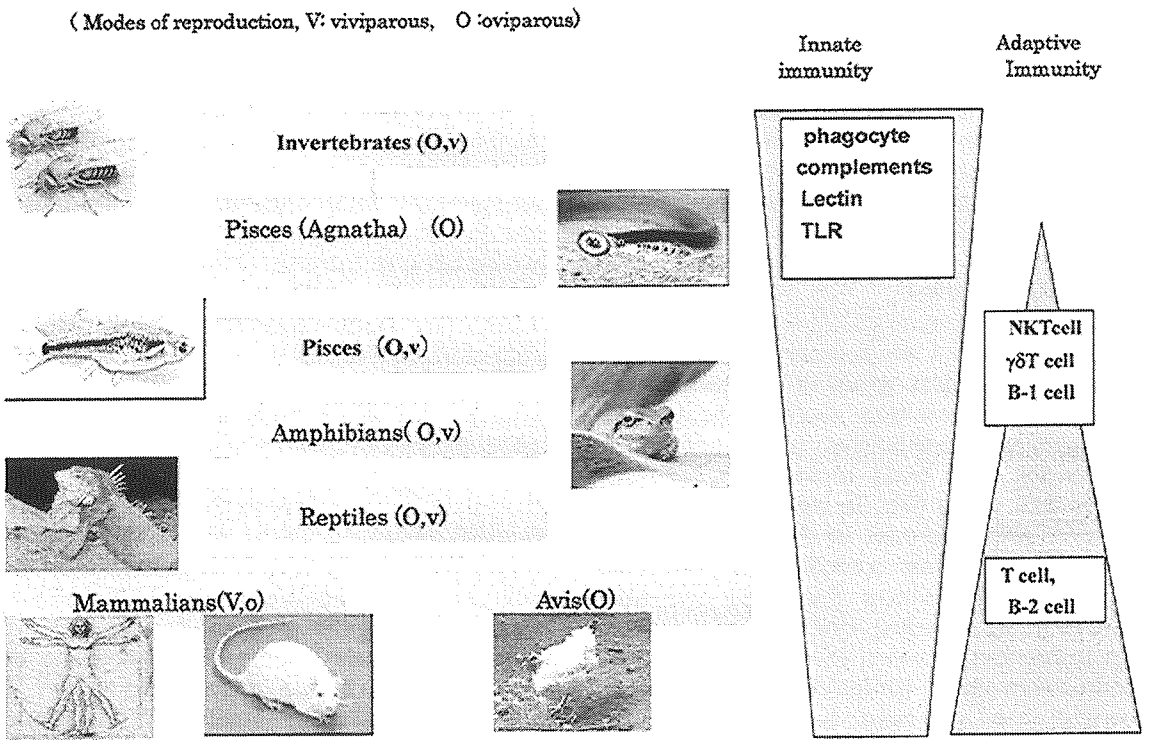

Figure 1 Modes of reproduction and evolution of immune system. Adaptive immune system mediated by genetically re-arranged immunglobulins and T cell receptors evolve at the level of jawed fishes. Though viviparity is not specific to mammalians, viviparous animals with adaptive immune system had to solve feto-placental surival against maternal immune recognition in utero.

cytes) against the NK sensitive human erythroleukemia cell line K562 [5]. Although the precise mechanism of cell recognition and contact-induced killing has not been clarified, their findings suggest the evolutionary conservation of an innate immune system that eliminates infected, mutated, and/or degenerated cells.

An innate immunity-dependent recognition of neoplasms has been reported in mammals. Complement-independent recognition and Toll-like receptor (TLR) mediated activation of host immune cells were observed against mutated virus-infected cells [6] without antigenic recognition. The human complement system protects an individual against substances of nonself origin, including xenografts and microbial pathogens. Human cells express the complement regulatory proteins, CD46 and CD55, thereby circumventing attack by C3, a major effector of complements. Seya's group reported that certain malignant cells, particularly those undergoing apoptotic stress, can activate the homologous complement, overcoming the regulatory actions of CD46 and/or CD55. They also identified a novel gene product, M161Ag, that converts human cells into targets for the homologous complement.

Members of the TLR family have been shown to be important in the activation of cells by a variety of microbial agents. Although TLRs have been considered to be important for defense against bacterial and fungal infections, recent studies have also proposed roles against viral infections including oncogenic viruses [7]. These findings suggest an evolutionary conservation of innate immunity in the surveillance of tumor cells by complement as well as TLRs (Fig. 1).

Neoplasias in fishes

Neoplastic disorders are common in fishes, which represent the largest group of vertebrates with over 20,000 documented species. Spontaneous, carcinogen/radiation-induced and viral-induced malignancies have been reported in both wild and domestic fishes [8]. On the other hand, neoplasms in several groups of fishes, such as the chondrichthyan (Class chondrichthyes) elasmobranches (sharks, skates and rays) and other primitive fishes including African lungfishes (Family protopteriae), bowfish (Family Amiidae), and chondrosteam fishes (Order Acipensiformes), are generally rare but have been reported. Grof attributed this rarity of tumors to their unique biology, such as the high ionic strength of elasmobranch tissues and antiangiogenic factors, and/or to a relative lack of observation and examination.

Neoplasms in amphibians and reptiles

Spontaneous neoplasia is not frequent in the three orders of Amphibia. However, tumors have been reported in most major organ systems with various etiologies, including viral infection, environmental contaminants, and genetic predisposition [9]. Anurans seem to have a greater frequency of spontaneous neoplasms than do urodeles and they respond to chemical carcinogens in a manner analogous to mammalian species [10].

Neoplasia is an important form of disease in saurians [11]. The organs most commonly affected by neoplastic disease are the hematopoeitic system, the hepatic system, the skin, and tumors of the musculoskeletal system. Hernandez-Divers et al. suggested that neoplasia should be considered as a significant differential diagnosis when presented with a lizard that has nonspecific clinical signs. Furthermore, recent years have seen an inexplicable increase in the frequency of an appalling disease in sea turtles: fibropapillomatosis, which is probably caused by a herpes virus and causes tumors to grow throughout the turtle's body [12]. Green turtle fibropapillomatosis is considered to be an increasingly significant threat to the survivability of this species.

Escape mechanisms employed by malignancies and concepti

In 1953, the famous British immunologist Sir Peter Medawar published an essay entitled "Some Immunological and Endocrinological Problems Raised by the Evolution of Viviparity in Vertebrates" [13], which became the most influential theory driving the development of the field of reproductive immunology over the next 50 years. He demonstrated the role of tissue antigens in the recognition and rejection of skin grafts between genetically differing individuals, and other researchers focused on the genetic basis of tumor transplant rejection in mice. These realizations led Medawar to recognize the truly paradoxical nature of the immunological relationship between the pregnant mother and her antigenically foreign fetus. He stated: "The immunological problem of pregnancy may be formulated thus: how does the pregnant mother contrive to nourish within itself, for many weeks or months, a foetus that is an antigenically foreign body?"

Having identified the problem, Medawar offered some possible solutions, proposing: "The reasons why the foetus does not habitually provoke an immunological reaction from its mother may be classified under three headings: (a) the anatomical separation of foetus from mother; (b) the antigenic

immaturity of the foetus; and (c) the immunological indolence or inertness of the mother".

Later, he developed an interest in the expression of stage-specific fetal and onco-fetal antigens, in the context of their potential for the generation of anti-cancer vaccines [14].

A crucial feature of the maternal–fetal relationship is that the two circulatory systems remain almost completely separate throughout the period of gestation. The original concept of "the embryo, quâ tissue homograft", is not therefore, in the strictest sense, appropriate. It is at the level of the placenta, and the fetal membranes, that tissue contact between the fetal graft and the maternal host is made. The placenta itself is provided with a continuous and unbroken outer barrier layer of trophoblastic tissue, which exists together with a variety of other biological forms of trophoblast situated at different anatomical locations within the pregnant uterus. The feto–maternal interface at the non-placental regions of the implantation site consists of the outermost of one of the extra-embryonic fetal membranes, which in man is trophoblastic in nature but in other species, notably laboratory rodents, is non-trophoblastic. The true fetal allograft of pregnancy is, therefore, the trophoblast and extra-embryonic membrane(s), both having direct cellular contact with the maternal uterine environment [15].

Recent advances in reproductive immunology have revealed more the detailed mechanisms involved in the maternal–fetal relationship and have shed light on why feto-placental units are not susceptible to attack by the maternal immune system (Fig. 2). Surprisingly, most escape mechanisms employed by mammalian concepti have shown striking similarities to those employed by human malignancies, as well as with those observed in experimental animals.

Burnet and Thomas proposed an immuno-surveillance hypothesis, which holds that the immune system can recognize and destroy nascent transformed cells. However, this hypothesis was soon abandoned due to the absence of strong experimental evidence supporting the concept. Recently, there has been an accumulation of experimental data supporting the old hypothesis of cancer immunosurveillance and indicating that it may function as a component of a more general process of cancer immunoediting. This process is responsible for eliminating tumors, sculpting the immunogenic phenotypes of tumors that eventually form in immunocompetent hosts, and facilitating tumor escape from immune destruction [16].

Non-classical HLA HLA-G and HLA-E

Both concepti and several types of malignancies are known to escape from the cytotoxic T cell (CTL) response and NK cell-mediated cell lysis by monomorphic (or oligomorphic) major histocompatibility complex class I molecules. Engagement by the T cell receptor (TCR) of a complex between the peptide and MHC (peptide-MHC) on antigen-presenting cells (APCs) is required for the development of T cells in the thymus, their survival as naïve cells in the periphery, and their activation and effector functions. In humans, there are six major groups of HLA (human leukocyte antigens): A, B, C, D, Dr, and Dq. They are categorized as classical HLA and they act as antigens when transplanted to recipients.

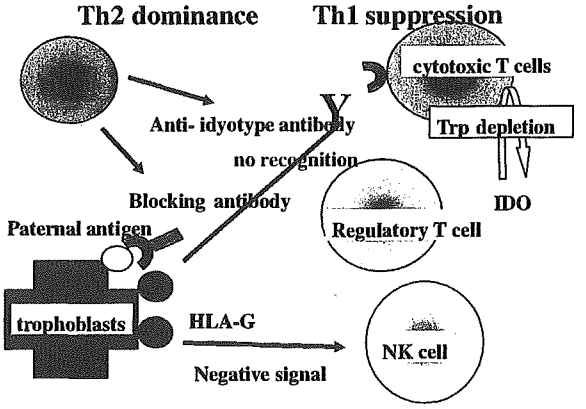

Figure 2 Immune responses to allogenic fetus.The mammalian fetus, except in instance in which the mother and the father are syngenic, will express paternally inherited antigen that are allogenic to mother. Protection of semi-allogenic fetus against maternal immune system involves several mechanisms. These mechanisms share common properties employed by malignancies.

HLA-G is a non-classical MHC-I molecule that is primarily expressed at the fetal–maternal interface, where it is thought to play a role in protecting the fetus from the maternal immune response. HLA-G binds a limited repertoire of peptides and interacts with the inhibitory leukocyte Ig-like receptors,LIR-1 and LIR-2, and possibly with certain natural killer cell receptors. The expression of the non-classical HLA-G molecule on various cancer cell lines and clinical samples including renal clear cell carcinoma [17], ovarian carcinoma [18], melanoma [19], and breast carcinoma [20] has been reported. The expression of HLA-G in cancer represents a strategy employed by tumors to avoid immune destruction. Hansel et al. reported recently that the up-regulation of HLA-G is associated with a neoplastic progression of pre-malignant and malignant lesions of colorectal epithelial tumors [21]. On the other hand, Dutta et al. have reported an absence of HLA-G but frequent expression of another non-classical antigen, HLA-E mRNA, in primary gastric carcinomas [22]. Expression of these non-classical HLAs in cancer represents a strategy employed by tumors to avoid immune destruction. Indeed, this non-classical HLA class I molecule suppresses various immune cell functions through binding to inhibitory receptors.

It is believed that MHC-deficient tumor clones can escape T-cell immune responses, but are in theory more susceptible to NK-cell-mediated lysis. However, if they express non-classical HLA class I molecules, they escape via NK inhibitory receptors [23]. The up-regulation of HLA-G gene transcription by tumor environmental factors such as cytokines, stress, and agents used in chemotherapy such as demethylating molecules has been reported as has the subsequent evasion of malignant cells from the antitumor immune response.

Surprisingly, increased expression of HLA-G was observed in endomyocardial biopsy specimens obtained from heart transplants, without chronic rejection [24]. Taken together,these findings suggest that HLA-G may be a master-key molecule for evading allograft rejection that has been gifted by evolution.

Indoleamine 2,3-dioxygenase (IDO)

Indoleamine 2,3-dioxygenase (IDO) is an enzyme that degrades the essential amino acid tryptophan. In murine experimental systems, the expression of IDO correlated with reduced T cell-mediated responses in autoimmune diseases, cancer, organ and tissue transplant rejection, and pregnancy [25]. Pregnant mice treated with IDO inhibitors experienced fetal resorption associated with extensive inflammation complement deposition and hemorrhagic necrosis at the maternal–fetal interfaces. The fetal allograft rejection was completely allo recognizing T cell-dependent because it was not observed in mice carrying syngeneic fetuses nor in T cell-deficient mice. These data show that IDO activity protects the fetus by suppressing T cell-driven local inflammatory responses to fetal alloantigens. IDO is expressed in dendritic cells as well as macrophages. In human placental tissue, Honig et al. localized the expression of IDO in syncytiotrophoblasts and endothelial cells as well as extravillous trophoblasts (EVT) by immunohistochemical techniques. They reported that EVT is the main source of IDO with blocking experiments [26].

In cancer tissues, increased expression of IDO has been reported in plasmocytoid dendritic cells in tumor drainage lymphocytes [27] as well as in carcinoma cells themselves such as in cervical carcinoma [28], esophageal carcinoma [29], and estrogen receptor negative breast cancer cell lines [30]. The expression of IDO has recently been reported to be controlled by a transcription factor, Bin1, which is attenuated in many human malignancies. Further, mouse knockout studies have shown that Bin1 loss elevates the STAT1- and NF-kappaB-dependent expression of IDO, driving the escape of oncogenically transformed cells from T cell-dependent antitumor immunity [31].

Release of immunosuppressive cytokines

Both malignancies and concepti secrete regulatory molecules including cytokines and prostanoids to evade both local and systemic immune responses. Cytokines are low molecular weight proteins that use their ability to act as intercellular communicators to regulate the immune response. A variety of cell types, principally T-helper lymphocytes and macrophages, can secrete cytokines in response to various stimuli. The functions that cytokines induce can both turn on and turn off particular immune responses.

Many types of malignancies have taken advantage of the regulatory role of cytokines to down-regulate appropriate immune responses targeted at destroying them. Cancers secreting immunosuppressive cytokines have been reported to be more aggressive and are associated with higher metastatic rates, and shorter survival periods of the hosts. For example, interleukin-6 (IL-6), interleukin-10 (IL-10), vascular endothelial growth factor (VEGF), Granulocyte colony stimulation factor (G-CSF), and transforming growth factor-beta (TGF-β) secreted by cancers often induce generalized

and specific inhibition of immune responses. We have reported immuno-regulatory roles of placenta-derived G-CSF in pregnant subjects as well as its suppression on autologous tumor killing activity in patients with ovarian carcinoma [32,33]. Of particular importance is the finding that most lymphocytes, including NK cells, NKT cells, and alpha–beta or gamma–delta T cells accumulating at tumor sites [34] produce IL-4, IL-10, and TGF-β and possibly inhibit CTLs and T helper 1 (Th1) cell responses, as observed in decidual intraepithelial lymphocytes. Gamma–delta T cells with regulatory roles were independently discovered by the author and Seo in deciduas and tumor tissues [35–37].

Secretion of prostaglandins

Prostaglandins secreted from cancer tissue may affect the host immune response as observed in decidual placental units. Prostaglandins affect cell differentiation, proliferation, and apoptosis, as well as target cell interaction. In human tissues, monocytes and macrophages are the cells responsible for prostaglandin production. Monocytes and related cells play a key role in regulating the interleukin cascade leading to T cell proliferation and finally to the immune response. They can either amplify the response by producing interleukin 1 or shut it down mainly by releasing prostaglandin E2. In vitro, prostaglandins have been shown to inhibit lymphocyte mitogenesis, cytolysis, and antibody production. In cancer tissues, increased expression of cyclooxygenase-2 (COX-2) has been reported in various human neoplasms including malignancies of the gastrointestinal tract and reproductive organs,in brain tumors, and in cancers of cancers of the head and neck. Increased production of prostanoids and subsequent local or systemic immune suppression are attributed to poor prognosis in patients with COX-2 positive tumors.

A hypothesis presented by Tawfik explains PGE2 as a primary suppressor molecule in decidual tissue. They observed a high concentration of PGE2 and PGF2alpha in cultured murine decidual cells, and transferable immuno-suppressive activity with reversal by indomethacin, an inhibitor of prostaglandin synthesis [38]. In humans, Kvirkvelia et al. observed selective expression of COX-2 in the syncytiotrophoblast of the chorionic villi and demonstrated an immunosuppressive effect of PGE2 on CTLL-2 cells via the EP4 receptor using highly selective antagonists [39]. Recently, trophoblast, uterine epithelium, and endometrial glands have all been shown to express haematopoietic-type prostaglandin (PG) D_2 synthase (hPGDS) and to recruit Th2 cells and Tc2 cells from peripheral circulation to the materno-fetal interface (implantation site) by chemoattraction mediated by PGD_2 and its receptor, CRTH2 [40].

Lack and aberrant expression of co-stimulation molecules

The presence of inadequate, inappropriate, or inhibitory T cell co-stimulatory pathway signaling has been shown to restrict a host's ability to generate productive immune responses against carcinomas and concepti. The expression of B7 on the surface of a cell is the co-stimulatory signal necessary to allow for the cytolytic CD8+ T cell attack on the targets. The co-stimulation results from an interaction of the CD28 molecule on the T cell surface with its ligand, B7, on the surface of an antigen-presenting cell (APC). The B7 display renders target cells capable of effective antigen presentation, leading to their eventual eradication. Related to this, the co stimulatory ligand, B7-H1 (PD-L1), has recently been implicated as a negative regulator of antitumoral T cell-mediated immunity. The expression of B7-H1 is normally restricted to macrophage-lineage cells, providing a potential co-stimulatory signal source for the regulation of T cell activation. In contrast, an aberrant expression of B7-H1 by tumor cells has been implicated in the impairment of T cell function and survival, resulting in defective host antitumoral immunity. B7-H1 expressed by tumor cells has been shown to enhance apoptosis of activated tumor-specific T cells in vitro [41]. Consistent with these observations, an in vivo monoclonal antibody blockade of B7-H1 has been shown to potentiate antitumoral responses in several murine cancer models.

The expression of B7-H1 has been described in a number of human malignancies including non-small cell lung carcinoma [42], ovarian carcinoma, and renal cell carcinoma [43].

In placental tissues, the mRNA for B7-H1 (PD-L1) and B7-DC (PD-L2) was reported to be highly expressed throughout gestation [44,45]. Petroff et al. localized an abundant expression of B7-H1 in cytototrophoblasts and syncytiotrophoblasts, as well as in extravillous cytotrophoblasts, all of which were juxtaposed to maternal blood and decidual tissue. They proposed the existence of a mechanism for active suppression of the maternal immune system that still maintains the ability to protect against foreign pathogens [46].

A second receptor for B7 on Tcells is cytotoxic lymphocyte antigen-4 (CTLA-4), the engagement of which provides an inhibitory signal that is imperative for the negative regulation of the immune

system [47] Constitutive expression of CTLA-4 mRNA and protein was reported in fetal tissues at the maternal–fetal interface throughout gestation [48], as well as in various cancer tissues [49].

Th2 predominance in pregnant women and cancer patients

Helper T cell responses differ between the two subpopulations, Th1 and Th2, according to differences in their cytokine expression profiles. IFN-γ, secreted from Th1 cells, is known to induce the differentiation of Th0 cells to Th1 cells and to inhibit the proliferation of Th2 cells. IL-4 and IL-10, secreted from Th2 cells, are known to induce the differentiation of Th0 to Th2 cells and to inhibit the function of Th1 cells [50]. It is widely believed that a Th2-dominant cytokine environment is necessary for successful pregnancy, while the actions of Th1 cytokines are thought to be detrimental to the fetus [51,52].

In cancer bearing patients, Th1 immunity plays a key role in the host defense against tumors, while a Th2-dominant environment has been shown to favor tumors [53–56].

The regulation of Th1/Th2 responses has been shown to be critically important for antitumor immune responses, such as the inhibition of tumor growth and metastasis, and increased survival rates in experimental animals. In humans, it was reported that the T cell responses shifted from Th1- to Th2-dominant status depending on the malignancy stage [57].

CD4+ CD25+ T regulatory cells (Treg)

CD4+ CD25+ T regulatory cells (Treg) are thought to be a functionally unique population of T-cells. They suppress antigen-specific immune responses and are important for allograft tolerance and suppression of autoimmune responses as well as for successful pregnancy both in mice [58] and humans [59,60]. These data support the concept that normal pregnancy is associated with an elevation in the number of Treg cells which may be important in maintaining materno-fetal tolerance.

In cancer immunology, experimental animal models and patients with various neoplasms have shown an increase in circulating Treg cells accompanied by their increased involvement in down-regulation of effector functions against tumors, resulting in T-cell dysfunction in cancer-bearing hosts [61–66]. Of particular interest, is the finding that the population of Tregs in tumor-infiltrating lymphocytes (TILs) of patients with advanced gastric cancer with a poor prognosis was significantly higher than that of TILs in patients with early gastric cancer.

The increase of Treg cells during pregnancy is partially attributed to estrogen [67]. The peripheral blood Treg population peaks during the second trimester and then declines in the postpartum period.Possible roles of placenta-derived endocrine factors or chemokine/cytokine control on local accumulation have been suggested but have not been adequately documented. Treg cells specifically express the chemokine receptors CCR4 and CCR8 and respond to the chemokines macrophage-derived chemokine (MDC/CCL22), thymus and activation-regulated chemokine (TARC/CCL17), I-309/CCL1, and the virokine vMIP-I, which are agonistic ligands of these receptors. As reported by the authors and other researchers, TARC, MDC, and other fraktalkines are produced in the villous and extra-villous trophoblasts in human placentae [68]. These chemokines, since they are produced by various human malignancies, are likely candidates to accumulate Treg in cancer tissues.

Trade-off in evolution and viviparity

The concept of a trade-off represents a key paradigm in evolutionary medicine. Over the last few decades there has been considerable effort to introduce an evolutionary perspective to biomedical research in the context of examinations, encouraging both researchers and clinicians to ask questions pertinent to the origin, and not simply the management, of a disease [69,70].

The concept of trade-off is common in economics, denoting choices made to accept less of one thing in order to acquire more of another. For example, when one is allocating (limited) funds, the trade-off usually involves reduced spending for some purposes to allow spending for other, perhaps more urgent purposes [71]. This concept can be readily extended beyond decisions involving money to human behaviors and non-human events. During evolution based on Darwinian selection, trade-offs were important drivers of biological events such as host–parasite relationships between humans and microorganisms [72,73], mate-choice decisions by female crickets [74], and the coexistence of specialists and generalists on an ecological timescale [75].

As individuals are always at risk of death, selection favors early reproductive opportunities over the potential for later ones. Accordingly, selection is never more efficient than at the age of commencement of reproduction, and this efficiency declines thereafter. Thus, traits that have beneficial effects

in early life will tend to spread, even if inseparately coupled to deleterious late effects [76]. Crespi and Semeniuk hypothesized that parent–offspring conflict over the degree of maternal investment has been one of the main selective factors in the evolution of the vertebrate reproductive mode [77]. It is proposed that in all organisms where parents and their offspring are not genetically identical, conflicts of interest will arise between them over the level of parental investment [78]. Their hypothesis is supported by several lines of evidence: the high number of independent origins of viviparity, matrotrophy (direct maternal–fetal nutrient transfer), and hemochorial placentation (direct fetal access to the maternal bloodstream); the extreme diversity in physiological and morphological aspects of viviparity and placentation, which usually cannot be ascribed adaptive significance in terms of ecological factors; and the divergent and convergent patterns in the diversification of placental structure, function, and developmental genetics. Furthermore, embryos and fetuses, like neoplasms, actively manipulate their interaction with the mother, thereby garnishing increased maternal resources. For the mothers, each of her genes has an equal probability of being present in each offspring, so her best strategy involves allocating nutrients to each offspring over her lifespan to maximize the aggregate reproductive success of her descendants. For offspring, the effects of genes are expected to be more selfish such that offspring are selected to seek greater investment from the mothers than she is selected to provide. Thus, intra-genomic conflict may occur when some elements in the genome – the so-called selfish – produce effects that enhance their own probability of replication or transmission at the expense of other elements within the same genome. For example, both protooncogenes and tumor suppressor genes have played crucial roles in cellular functions across hundreds of millions of years of evolution, and tumor suppressor genes have been shown to carry out policing functions in invertebrates as well as in vertebrates [79]. Summers et al. proposed that both parent–offspring and intra-genomic conflicts apparently favor the evolution of alleles that promote cancer [80].

Conclusions

The principal functions of the immune system in long-lived metazoans are supposed to be the defense against microbial pathogens and the surveillance and clearance of aberrant components of self. Viviparity requires immune suppression in pregnant females to protect their concepti from immunological attacks. For transformed cells, genes employed by feto-placental units allow them to escape detection by the host immune system. Given these findings and hypotheses, the author propose that the evolution of viviparity resulted from a trade-off that increased susceptibility to malignancies in exchange for survival of the feto-plancental unit against the maternal immune system. This hypothesis would also explain the paucity of malignancies among invertebrate animals. Our ancestors may have opted for the big-ticket purchase of successful viviparity while paying for it in part with a somewhat compromised immune defense system.

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Rembrandt's Bathsheba, possible lactation mastitis following unsuccessful pregnancy

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Summary Deformity of the left breast and axilla observed in Rembrandt's famous painting "Bathsheba at her toilet" (1654, Louvre Paris) has been discussed by several researchers. Proposed diagnoses were breast cancer and abscess due to tuberculosis. The present article reviews previous articles written concerning the left breast abnormalities of Bathsheba and carefully examines other works of Rembrandt modeled by Hendrickje and painted around 1654. Previous diagnosis of breast cancer and tuberculous mastitis is less probable. Because Hendrickje survived for more then 9 years after the painting and in other works modeled by Hendrickje shows no signs of cachexia or permanent changes in the left breast. The most likely diagnosis of the left breast deformity of Bathsheba is a sequela of lactation mastitis abscess following miscarriage or premature childbirth without breast feeding.

A case study and proposed diagnoses

Rembrandt van Rijn (1606–1669) born in Leiden was one of the most famous and leading representatives of the Dutch School of painting and was especially talented in the use of light and shadow [1]. He left correct paintings of medical scenes known as "Anatomy lesson by Dr. Nicholaes Tulp" (1632, The Hague, Mauritshuis museum) and "The anatomy lesson of Dr. Joan Dejiman" (1656, Amsterdam Historisch Museum).

Other than medical paintings, he recorded clinical findings of basal cell carcinoma in "Man in oriental costume" (1632, New York metropolitan Museum) [2], signs of ageing including brow and eyelid ptosis, rosacea and temporal arteritis in his self-portrait (1659, National Gallery of Art, Washington, DC) [3].

In 1983 Braithwaiste and Shugg suggested that Rembrandt's famous painting of "Bathsheba at her toilet" (1654, Louvre Paris) showed clinical signs of advanced left breast carcinoma based upon skin discolouration, distortion, axillary fullness and peau d'orange appearance [4]. The breast cancer hypothesis was presented independently by Dymarskii in 1984 [5].

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It is generally accepted that Bathsheba was painted in 1654 modeled by Hendrickje Stoffels. In 1654, Hendrickje was 28 years old. She was Rembrandt's de facto wife as of 1649 and had a pregnancy in 1652. She became pregnant again and delivered their daughter Cornelia in 1654 October.

No records on health of Hendrickje until her premature death on 21 Jul 1663 (37 years old).

In 2000, Bourne proposed on alternative diagnosis of an infective process such as tuberculous mastitis or less likely chronic lactational breast abscess [6]. He discussed that if the body of model was Hendrickje, she could hardly have lived 9 years with advanced breast cancer without any effective treatment. Possibly she had a chronic inflammatory condition, either tuberculous mastitis and less likely lactational breast abscess.

Other works by Rembrandt modeled by Hendrickje

We can see other works by Rembrandt modeled by Hendrickje. Rembrandt left several oil paintings modeled by Hendrickje around 1654, including "Hendrickje Stoffels" (1655, Paris Musee Louvre) "Woman in a Doorway" (1656, Berlin Gemeldegalerie) and famous "Hendrickje bathing" (1655, London National Gallery). No paintings show the naked left breast of the model. But we can see a healthy slightly obese woman at 25–30 years, and observe no remarkable signs of cachexia or chronic consumption.

Further, we can see several female nude etchings (1658, New York, Pierpont Morgan Library) and a precise drawing "Hendrickje in the Artist's Studio" (1654, Oxford, Ashmolean museum). We can observe frontal view of the left breast in 3 etchings in New York and left posterior view in Oxford drawing. Neither etchings nor drawing show deformities or distortions such as dimpling or peau d' orange appearance and axillary lymph node swelling.

Discussion

Taken together, if the model of Bathsheba was Hendrickje and she suffered from left breast disease at the time of the painting, it must be a benign and possibly reversible change. Because Hendrickje delivered Rembrandt's daughter Cornelia in 1654 and survived for 9 more years after the delivery and painting of Bathsheba. If the left mammary changes were caused by breast cancer she would not have survived so long. As Bourne pointed out, the mean survival of breast cancer patients was 2–3 years before the application of modern surgical treatment, radiation and/or chemotherapy.

Diagnosis of tuberculous abscess looks less probable. Because breast involvement of tuberculosis is a rather rare complication. If the model had suffered from tuberculosis, she must show some signs of chronic consumption and hardly had a chance becoming pregnant. Further, untreated tuberculous abscesses persist for several years and result in permanent deformity of the breast. As we cannot see any changes in the Oxford drawing of 1654 and in the New York etchings of 1658, we need to abandon the possible diagnosis of tuberculous abscess and benign breast tumours including fibroma.

We present as a possible alternative, chronic lactational breast abscess and mastopathy. Even in the 21st century Japan and other developed countries, lactating women are often experience mastitis. However they can be effectively treated by the oral administration of antibiotics. In serious cases, they also can be treated by bromocriptin administration that inhibits lactation.

It is possible that lactating women in the mid 17th century had a higher prevalence of mastitis and subsequent breast abscess. In the acute phase of mastitis, redness and swelling are remarkable but they become less observable in the chronic phase and after abscess formation. Hendrickje delivered her second child Cornelia in October of 1654, she had a chance of breast-feeding and subsequent infection. However, if Bathsheba was painted in 1654, birth of Cornelia looks less probable as the cause of chronic breast abscess formation. Thus, we consider it was painted early 1654, before the conception of Cornelia or during the first trimester of pregnancy. As Hendrickje had been reported to become pregnant in 1652, she had the opportunity for lactation and related inflammation. The outcome of Hendrickje's first pregnancy is not known. As there are no record concerning Rembrandt's children except for Titus (borne in 1641, the only living son between Rembrandt's first wife Saskia) and Cornelia, Hendrickje's pregnancy in 1652 possibly resulted in miscarriage or neonatal death due to premature delivery.

Our hypothesis explains why Bathsheva shows no evidence of abdominal striae, which can often be observed in multiparous women. Premature labour or miscarriage even in the first trimester of pregnancy never leave abdominal striae but can induce lactation and lactation without feeding is a major

cause of subsequent breast milk retention and inflammation.

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The incidence of pre-eclampsia among couples consisting of Japanese women and Caucasian men

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Abstract

Recent data from Hiby (2004) have suggested that a combination of maternal killer immunoglobulin receptor (KIR) AA genotype and fetal HLA-C2 genotype increases the risk of pre-eclampsia. Different human populations have a reciprocal relationship between KIR AA frequency and HLA-C2 frequency. Japanese people have highest frequency of KIR-AA alleles and lowest frequency of HLA-C2 alleles. However, Caucasians have a moderate frequency of KIR-AA and HLA-C2 alleles. If this hypothesis is correct, the incidence of pre-eclampsia among couples consisting of Japanese women and Caucasian men should be higher than that among couples consisting of Japanese women and Japanese men. Therefore, we investigated the incidence of pre-eclampsia among 324 couples consisting of Japanese women and Caucasian men. The incidence of pre-eclampsia in these couples consisting of Japanese women and Caucasian men was similar to that in Japanese women and Japanese men. Our data do not support that of Hiby et al. [Hiby, S.E., Walker, J.J., O'Shaughnessy, K.M., Redman, C.W.G., Carrington, M., Trowsdale, I., Moffett, A., 2004. Combinations of maternal KIR and fetal HLA-C genes influence the risk of pre-eclampsia and reproductive success. J. Exp. Med. 200, 957–965], although we did not check the haplotypes for HLA-C and KIR.

Keywords: Etiology; HLA-C; KIR; Pre-eclampsia; Human population

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1. Introduction

A number of hypotheses on the etiology of pre-eclampsia have been reported (Roberts et al., 1989; Arngrimsson et al., 1990; Perry and Martin, 1992; Meekins et al., 1994; Robillard et al., 1994; Zhou et al., 1997; Dekker et al., 1998; Redman et al., 1999; Koelman et al., 2000; Saito and Sakai, 2003). One commonly discussed hypothesis is the immunogenetic maladaption hypothesis (Dekker et al., 1998; Robillard et al., 2002; Koelman et al., 2000; Saito and Sakai, 2003; Chaouat et al., 2005). Immune recognition of fetal (paternal) antigens is suggested by the increased risk of pre-eclampsia in first pregnancies (MacGillivray, 1983; Skjaerven et al., 2002) and in multiparous women after changing partners (Robillard et al., 1999; Li and Wi, 2000; Trogstad et al., 2001). There is also an increased risk in women who have received donated gametes, such as artificial donor insemination (AID) (Hoy et al., 1999), oocyte donation and embryo donation (Soderstrom-Anttila et al., 1998). These findings suggest that maternal tolerance to paternal antigens is important for the maintenance of pregnancy, and immunogenetic maladaptation of tolerance system might induce pre-eclampsia. Extravillous trophoblasts (EVT) express four unique class I MHC molecules: HLA-G, HLA-E, HLA-F and HLA-C (Kovats et al., 1990; Ishitani et al., 2003; King et al., 1996). Only HLA-C is polymorphic, so paternal HLA-C on EVT can be recognized by killer immunoglobulin receptors (KIR) on maternal NK cells (Moffett-King, 2002). Recently, Hiby et al. (2004) reported interesting data showing that the combination of maternal KIR-AA, which has no activating receptors, and the fetal HLA-C2 group is associated with pre-eclampsia. They showed also that additional activating KIRs decrease the incidence of KIRs pre-eclampsia when the fetus has an HLA-C2 allele.

Recent data demonstrate that populations with a high KIR-AA genotype frequency have a low frequency of HLA-C2 alleles and vice versa (Williams et al., 2002; Yawata et al., 2002; Norman et al., 2001; Crum et al., 2000; Cook et al., 2003; Toneva et al., 2001; Rajalingam et al., 2002; Whang et al., 2003; Wang et al., 1997). Hiby et al. (2004) hypothesized that the KIR-AA/HLA-C2 combination in a given population would be selected against by deleterious effects such as pre-eclampsia. Japanese people have the highest frequency of KIR-AA genotype at around 60%, and the lowest frequency of HLA-C2 genotype at around 9% (Yawata et al., 2002). Conversely, Australian aborigines and New India have the lowest frequency of KIR-AA genotype and highest frequency of HLA-C2 genotype (Norman et al., 2001; Cook et al., 2003; Rajalingam et al., 2002). If the hypothesis of Hiby et al. (2004) is correct, the incidence of pre-eclampsia in couples consisting of Japanese women and Australian aborigine or New India men should be high. However, such couples are very rare. On the other hand, the number of couples consisting of Japanese women and Caucasian men has been increasing. Caucasians have moderately high frequencies of KIR-AA genotype and HLA-C2 genotype. The frequency ratio of the HLA-C2 genotype is 30–35%, which is three to four times higher than that in Japanese (Williams et al., 2002; Hiby et al., 2004). Therefore, we investigated the frequency of pre-eclampsia in couples consisting of Japanese women and Caucasian men.

2. Materials and methods

2.1. Study patients

Between January 2002 and October 2005, a total of 328 couples consisting of Japanese women and Caucasian men delivered infants at our hospital. There were 332 infants (324 singletons and four sets of twins). We selected the 324 singleton subjects, because twin pregnancy is one of the risk factors for pre-eclampsia. As a control, 36,829 singleton pregnant women were selected from the 2003 database of the Japan Society for Obstetrics and Gynecology. In this database, the nationalities of patients or husbands were not recorded, but the vast majority of cases (more than 98%) were couples consisting of Japanese women and Japanese men in Japan. Therefore, we used this database as the control.

Gestational hypertension was diagnosed by the following definitions: blood pressure (BP) levels >140/90 mmHg after 20 weeks of gestation. Gestational hypertension with proteinuria as indicated by a single albumin reading at least 30 mg/dl (a dipstick reading of 1+) after 20 weeks of gestation was diagnosed as pre-eclampsia. Statistical analysis was performed by Student's *t*-test.

3. Results

Table 1 summarizes the characteristics of couples consisting of Japanese women and Caucasian men, and the controls. There were no significant differences in age, parity, gestational weeks at delivery, maternal body weight at delivery, and treatment for sterility between the test couples and controls.

We observed gestational hypertension in 2.16% of couples consisting of Japanese women and Caucasian men, and in 3.84% of controls (Table 2). The frequencies of gestational hypertension among nulliparous and muliparous test couples were the same as that among controls. The relative risks of the incidence of gestational hypertension among test samples were 0.68 in nulliparous, 0.27 in multiparous, and 0.56 in total couples. The 95% confidence intervals (CI) were 0.3–1.55 in nulliparous, 0.04–1.91 in multiparous, and 0.27–1.19 in total couples, respectively. The frequency of pre-eclampsia among test couples (1.54%) was slightly lower compared to that among controls (2.67%) (Table 2), although there was

Table 1
Characteristics of couples consisting of Japanese women and Caucasian men and controls

	Couples consisting of Japanese women and Caucasian men (n=324)	Control (n=36,829)	p-Value
Age (years)	32.2 ± 4.3	31.8 ± 4.1	0.743
Primipara	199 (61.4%)	21816 (59.2%)	0.426
Maternal body weight at delivery (kg)	62.8 ± 6.9	62.1 ± 7.2	0.825
Neonatal body weight (g)	3133.0 ± 519.6	3026.2 ± 502.5	0.693
Therapy for sterility	17 (5.2 %)	1593 (4.3%)	0.417

The data were shown as mean ± S.D.

Table 2
Frequency of gestational hypertention and pre-eclampsia of couples consisting of Japanese women and Caucasian men and controls

	Couples consisting of Japanese women and Caucasian men (n=324)	Control (n=36,829)	p-Value	RR (95% CI)
Gestational hypertension				
Nulliparous	6/199 (3.02%)	961/21816 (4.41%)	0.341	0.68 (0.3–1.55)
Multiparous	1/125 (0.8%)	452/15013 (3.01%)	0.149	0.27 (0.04–1.91)
Total	7/324 (2.16%)	1413/36829 (3.84%)	0.117	0.56 (0.27–1.19)
Pre-eclampsia				
Nulliparous	4/199 (2.01%)	681/21816 (3.12%)	0.369	0.64 (0.24–1.74)
Multiparous	1/125 (0.8%)	303/15013 (2.02%)	0.334	0.40 (0.06–2.85)
Total	5/324 (1.54%)	984/36829 (2.67%)	0.209	0.58 (0.24–1.4)

RR, relative risk; CI, confidence interval.

no significant difference between the two groups. We observed pre-eclampsia in 3.12% of nulliparous women and 2.02% of multiparous women in the control group. The frequencies of pre-eclampsia among nulliparous women and multiparous women in the test group were 2.01% and 0.8%, respectively. There were no significant differences in the frequency of pre-eclampsia between couples consisting of Japanese women and Caucasian men, and the control group (Table 2). The relative risks and 95% CI of the incidence of pre-eclampsia among test samples were 0.64 (0.24–1.74) in nulliparous, 0.40 (0.06–2.85) in multiparous, and 0.58 (0.24–1.40) in total couples.

4. Discussion

The report by Hiby et al. (2004) has had significant impact on consideration of the pathophysiology of pre-eclampsia. They pointed out that the combination of maternal KIR-AA genotype and fetal HLA-C2 genes is at increased risk for pre-eclampsia, and showed that different human populations have a reciprocal relationship between KIR-AA frequency and HLA-C2 frequency. Their hypothesis is attractive, but further studies are needed for verification. As one method of proving their hypothesis, an epidemiological study could be valid. A population with a high frequency of KIR-AA genotype and a low frequency of HLA-C2 would result in a low frequency (3–5%) of pre-eclampsia but, if women from a population with a high frequency of HLA-AA marry men from a population with a high frequency of HLA-C2, the prevalence of pre-eclampsia should be increased. The Japanese population has the highest frequency of KIR-AA (~60%) (Yawata et al., 2002; Hiby et al., 2004), while, Caucasian populations have a 3.5 times higher frequency of HLA-C2 genotype than Japanese. If hypothesis of Hiby et al. (2004) is correct, the prevalence rate of pre-eclampsia among such couples should show three- to four-fold increase.

However, our data have now shown that the incidence of pre-eclampsia among couples consisting of Japanese women and Caucasian men did not differ significantly from that

among couples consisting of Japanese women and Japanese men. In this cohort, considering the nulliparae as pre-clampsia is often a disease of first pregnancies, the prevalence of gestational hypertensive disorders of pregnancy among nulliparae was 5.03% in cases versus 7.53% in controls. The relative risk and 95% CI of pre-eclampsia among the test group were 0.64 (0.24–1.74) in nulliparous, 0.40 (0.06–2.85) in multiparous, and 0.58 (0.24–1.4) in total couples. They did not reach to three- to four-fold increase in prevalence which was calculated by the hypothesis of Hiby et al. (2004). Thus, our epidemiological study does not support that hypothesis, although we did not investigate individual KIR and HLA-C genotypes. Robillard et al. (1994) reported that the duration of sexual cohabitation effects the risk of gestational hypertension. Unfortunately, we could not obtain the information about the length of sexual cohabitation. The present findings suggest that further investigations of maternal KIR genotype and fetal HLA-C genotype, or duration of sexual cohabitation, are needed to confirm this intriguing hypothesisin pre-eclampsia.

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IDO expression on decidual and peripheral blood dendritic cells and monocytes/macrophages after treatment with CTLA-4 or interferon-γ increase in normal pregnancy but decrease in spontaneous abortion

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Recent data demonstrated that $CD4^+CD25^+$ regulatory T (Treg) cells and an enzyme called indoleamine 2,3-dioxygenase (IDO) mediate maternal tolerance to the fetus. Interestingly, Treg cells express the CTLA-4 molecule on their surface, and B7 (CD80/86) ligation by CTLA-4 enhanced IDO activity of dendritic cells (DCs) and monocytes by the induction of interferon gamma (IFN-γ) production. In this study, we studied the IDO expression on peripheral blood monocytes and decidual monocytes or DCs after treatment with CTLA-4/Fc fusion protein or IFN-γ using flow cytometry. IDO expressions on both peripheral blood DC and decidual DC and monocytes were up-regulated during normal pregnancy. On the other hand, both IDO expression on DC and monocytes after IFN-γ treatment or CTLA-4 treatment were decreased in spontaneous abortion cases. The expression of CD86 on peripheral blood and decidual monocytes and DC in spontaneous abortion cases was lower compared with those in normal pregnancy subjects. Also, IFN-γ production by decidual and peripheral blood mononuclear cells after CTLA-4/Fc treatment in spontaneous abortion cases was significantly lower than those in normal pregnancy subjects. These data suggest that CTLA-4 on Treg cells up-regulates IDO expression on decidual and peripheral blood DC and monocytes by the induction of IFN-γ production.

Key words: CTLA-4/dendritic cell/IDO/pregnancy/regulatory T cell

Introduction

A fetus is a semi-allograft to the maternal host, and T cells are aware of fetal alloantigens. Using T-cell receptor (TCR) transgenic mice, Tafuri *et al.* (1995) demonstrated that maternal H-$2K^b$-specific $CD8^+$ T cells were functionally tolerized by fetal H-$2K^b$ alloantigen, but this state lasted only briefly after parturition. Supporting evidence was obtained from studies in which pregnant mice carrying syngeneic or allogeneic fetuses were treated with a pharmacologic inhibitor of an enzyme called indoleamine 2,3-dioxygenase (IDO) (Munn *et al.*, 1998). IDO is expressed in specific populations of macrophages (Mφ) and dendritic cells (DCs), giant trophoblasts in mice and extravillous trophoblasts and villous trophoblasts in humans (Munn *et al.*, 1999, 2002; Hwu *et al.*, 2000; Sedlmayr *et al.*, 2002; Baban *et al.*, 2004; Honig *et al.*, 2004; Kudo *et al.*, 2004). These findings suggest that immunosuppressive Mφ and DC in decidua prevent maternal T-cell activation by depriving T cells of tryptophan. Serum tryptophan levels decrease from the first trimester of human pregnancy (Schrocksnadel *et al.*, 1996), suggesting that tryptophan metabolism protects the allogenenic fetus in humans by inducing maternal tolerance, although IDO-deficient mice produce litters of normal sizes at normal rates compared with wild mice (Baban *et al.*, 2004).

Recently, it has been reported that T-cell responses are regulated by $CD4^+CD25^+$ regulatory T (Treg) cells, and these Treg cells play a very important role in immunotolerance. $CD4^+CD25^+$ Treg cells express the CTLA-4 molecule on their surface, and CTLA-4 can enhance the IDO activity of DC and Mφ (Grohmann *et al.*, 2002; Fallarino *et al.*, 2003). IDO induction in Mφ or DC is one mechanism by which $CD4^+CD25^+$ Treg cells induce tolerance. Interestingly, recent data showed that $CD4^+CD25^+$ Treg cells are essential for the maintenance of allogeneic pregnancy in mice by the induction of maternal tolerance to fetal antigens (Aluvihare *et al.*, 2004; Zenclussen *et al.*, 2005). In human pregnancy, regulatory T cells increase in peripheral blood and decidua, and decidual $CD4^+CD25^+$ Treg cells express CTLA-4 on their surfaces (Heikkinen *et al.*, 2004; Sasaki *et al.*, 2004; Somerset *et al.*, 2004). However, it has not been reported whether CTLA-4 can induce IDO protein expression in DC or monocytes during pregnancy.

IDO expression is also up-regulated by interferon gamma (IFN-γ) treatment (Taylor and Feng, 1991; Munn *et al.*, 1999, 2004). IFN-γ is produced by decidual T cells and natural killer (NK) cells (Saito *et al.*, 1993; Jokhi *et al.*, 1994) and plays important roles in angiogenesis (Ashkar *et al.*, 2000). In this study, we checked the IDO expression in peripheral blood and decidual monocytes or DC after treatment with CTLA-4 or IFN-γ in normal pregnancy subjects. We further compared these effects in spontaneous abortion cases with those in normal pregnant subjects. Our data showed that the IDO expression on DC and