

Origin of anti-tumor immunity failure in mammals and new possibility for immunotherapy

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Summary There is now much evidence that tumors can be immunogenic. Tumor cells very often express antigens in a form recognizable by the host immune system, but most frequently without consequences on tumor progression. This has been shown in many experimental models and different experimental conditions. Immediate mechanisms for the escape of tumors from immune response are very similar with mechanisms for the escape of fetoplacental unit (as an allograft) from maternal immune response. Similarity between these two mechanisms is so significant that any randomness is banished. Mechanisms of anti-tumor immunity in mammals are substantially different in comparison with mechanisms of anti-tumor immunity in other classes of vertebrates. Moreover, type of most frequently tumors in non-mammalian vertebrates is also significantly different. Incidence of malignant tumors in non-mammalian vertebrates is significantly less than incidence of malignant tumors in mammals. These facts indicate that immune system of mammals during anti-tumor immune response is tricked with similarity between tumor cells and trophoblast or other placental cells. It may be a specific evolutionary approach in rendering of anti-tumor immunity failure in mammals, and new possibility for anti-tumor immunotherapy.

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BASIC MECHANISMS OF ANTI-TUMOR IMMUNITY FAILURE IN MAMMALS

Mechanisms for the escape of tumors from immune response include:

Downregulation of immune response by the tumor and/or immunocompetent cells and their products

Part of these mechanisms is established on several immunomodulatory/immunosuppressive cytokines like TGF- β , IL-10, and IL-6. High levels of TGF- β have been found in serum of patients in colorectal to the degree of tumor progression, and shown decrease after resection (1). In patients with disseminated melanoma, TGF- β levels in plasma are higher than in patients with local

melanoma (2). If tumor shown signs of regression, TGF- β levels in plasma are significantly lower. IL-6 levels in the serum of cancer sufferers are also commonly elevated. In patients with lung cancer, melanoma and breast cancer levels of serum IL-6 strongly correlate with tumor progression (3,4). Elevated serum IL-10 concentrations have also been frequently reported in patients with various solid tumors like melanoma, pancreatic carcinoma, ovarian tumors, and breast carcinoma (5). Tumor regression is very often associated with downregulation of IL-10 production. Kim et al. (6) showed that intralesional treatment with IFN- α induced tumor regression, associated with downregulation of IL-10 mRNA.

Prostaglandin, like PGE₂ also have important role in suppression of anti-tumor immunity. Tumor cells or tumor infiltrating monocytes produces sizable amounts of prostaglandin, which have strong immunosuppressive effects (7,8).

Many authors showed that Th₂ immunity is ineffective in anti-tumor response. Athwart, Th₁ immunity and CTL or NK cells which stimulated by Th₁ cytokines (IL-2, IL-12, IFN- γ , and TNF- α) shows very efficient anti-tumor

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effects. In patients with malignant tumors the balance between Th₁ and Th₂ cells are perturbed in account of Th₂ cells. T cell lines and clones derived from tumor infiltrating lymphocytes commonly possess the characteristics of Th₂ cells (secretion of IL-3, IL-4, IL-6, IL-10, and GM-CSF but not IL-2, IFN- γ , and TNF- α) (9). IL-10 and TGF- β may be synergistic in inducing or maintaining T cell suppression and in diverting T cell response from Th₁ to Th₂ phenotypes (10). In some models, successful immunotherapy of established tumor is associated with change in the balance of T cell subset from Th₂ to Th₁ phenotype (11).

Anti-tumor immunity in malignant tumor patient may be downregulated by shed molecules other than cytokines. These molecules include oncofetal antigens, cancer/testis antigens, ICAM-1, and MUC-1 molecules and many other proteins with nonspecific immunosuppressive effects (12,13).

Alterations in signal transducing CD3 molecules may cause resistance of Th₂ cells to activation-induced cell death, and susceptibility of Th₁ cells on this type of apoptosis (14). Tumor cells and tumor infiltrating macrophages may be to induce alterations in signal transducing molecules. Aoe et al. (15) described decreased CD3 ζ expression even on freshly isolated tumor cells cocultured with tumor-derived macrophages. Expression of CD3 ζ molecules is linked with metabolisms of H₂O₂ in immunocompetent cells. Chronic inflammatory conditions in advanced cancer will alter the REDOX potential of macrophages, causing them to exert an immunosuppressive effector the host immune system via secretion of factors such as H₂O₂. These factors will rapidly shut off the effector function of CTL and NK cells (16).

Another mechanisms for the escape of tumor from immune response are mediated by process downregulation of immune response by apoptosis induction and anergy induction of immunocompetent cells. Fas-ligand found in 100% lung carcinoma cell lines (17). Melanoma cells commonly express fas-ligand, and induce apoptosis of fas+ susceptible T cell (18). In most case, magnitude of T cell apoptosis correlated with plane of tumor cells fas-ligand expression and tumor progression. Besides fas-ligand, expression of proteins like MUC-1 on tumor cells, also mediate in T cell apoptosis (19). Anergy induction is antigen-specific and is an early event associated with tumor progression. Stimulating T cells via TCR can induce T cells anergy in the absence of costimulation, or by partial agonist ligands or by preventing proliferation of responding cells by neutralizing the autocrine IL-2 pathway (20). Vaccination of mice with tumor peptides in the absence of any 'help' may itself also result in the induction of anergy, with the result that the immunized mice could no longer reject tumors that even non-immunized mice could (21).

Altered expression of MHC and/or tumor antigens by tumor cells (absence of expression of MHC antigens and expression nonclassical MHC antigens like HLA-G)

Poor prognosis of malignant disease has been documented in association with HLA loss and there may be a higher frequency of selective loss of HLA class I specificities in metastases compared to primary lesion (22). For example, in breast cancer, total class I loss was found in >50% of patients, with a further 35% showing selective losses and only 12% tumor retained full HLA class I expression (23). A common reason for decreased class I expression is the loss of peptide transporter gene expression. In the absence of TAP, antigenic peptides derived from the tumor cannot be presented. TAP defects may be corrected by treatment with IFN- γ (24). Transfection of IFN- γ genes into HLA deficient small lung cancer cells resulted in HLA upregulation and subsequent recognition of the tumor by MAGE-3 specific CTL (25). Tumors lacking MHC class I expression and therefore capable of escaping CTL response might become susceptible to anti-tumor activity based on NK cells. In melanoma patients, tumor variants no longer expressing MHC class I were indeed found to be susceptible to lysis by autologous NK cells (26). Tumor cells can express unusual forms and number of MHC antigens like HLA-G and HLA-C. These antigens may mediate inhibition of antigen-specific lysis by CTL and antigen-nonspecific lysis by NK cells (27). Moreover, HLA-G can mediate upregulation tumor-protective cytokines like TGF- β and IL-10 (28).

Altered expression of adhesion or accessory molecules by tumor and/or dendritic cells

Anti-tumor responses are commonly triggered by presentation of tumor antigen to T cells by host antigen presenting cells (APCs). If there are compromised in their function, anti-tumor immunity will be strongly affected. APCs in the infiltrates of human colorectal carcinoma were MHC class II+ essentially failed to express the costimulatory molecules CD80 or CD86 (29). Presentation of tumor antigen by HLA-DR in the absence of costimulation might induce anergy in the responding tumor specific T cells. APCs from progressing melanoma had depressed CD86 expression and secreted IL-10 and induce anergy in CD4+ cells, whereas APCs from regressing melanoma secreted IFN- γ and IL-12 and did not induce anergy in CD4+ cells (30). Tumor cells can express costimulatory molecules. High expression of CD86 molecule may be associated with selective stimulation of Th₂ cells and increased IL-4 production and suppression of anti-tumor immunity (31). High expression of CD80 molecules is associated with selective stimulation of Th₁

immunity and tumor regression. Tumor cells from tumor in progression or metastases showed poorly expression of CD80 and CD40 costimulatory molecules, while expression of CD86 molecule shows variable models of expression (32). Cytokine produced by TIL without costimulatory signals may stimulate tumor growth. For example, IL-10 produced by TIL or melanoma cells may operate as a growth stimulating factor for melanoma as well as reducing expression of HLA and adhesion molecules (33).

BASIC MECHANISMS OF ANTI-TROPHOBLAST IMMUNITY FAILURE

Mechanisms for the escape of fetoplacental unit (as an allograft) from maternal immune response include:

Downregulation of maternal immune response by the trophoblast and/or decidual immunocompetent cells and their products

Cytokines like TGF- β , IL-10 and IL-6 have very important role in mechanisms of trophoblast protection from maternal immune response. TGF- β strongly inhibits secretion of cytokines like IL-1 β , IL-2. TNF- α and TNF- β , and significantly decelerates rejection of any allograft (34). Moreover, TGF- β is a factor of promotion of Th₂ type immune response and upregulatory factor of Th₂ cytokines secretion (35). In patients with recurrent miscarriages past, level of trophoblast and decidual production of TGF- β ₂ is significantly less than in healthy pregnant women. TGF- β ₂ treatments of allopregnant mice in fetal resorption prone combination (CBA/JxDBA/2J) are associated with common rate of fetal resorption (35). Trophoblast and immunocompetent cells associated with decidua are very active in secretion of IL-6. High serum level of this cytokines in healthy pregnant women is usual, but pregnant women with habitual spontaneous abortion past show significantly less serum level of IL-6 (36). IL-6 stimulate trophoblast cells proliferation and theirs sensibility on other cytokines influence (37,38). IL-10 is cytokine, which suppress secretion any other cytokines, even cytokines from Th₂ group. Domination of Th₂ type of immune response under IL-10 influence is result of IL-10 antiapoptotic effect on Th₂ cells (39,40). Moreover, IL-10 downregulate trophoblast cells expression of MHC class I molecules, and T and NK cells expression of IL-2 receptors. After *in vitro* pretreatment with IL-10, T lymphocytes are in anergy state with basal production of IL-2 (39,40). Healthy pregnant women show significantly higher serum level of IL-10 than women after abortion (36).

Decidua and trophoblast are most important sources of synthesis and secretion of prostaglandin. Role of

prostaglandin in pregnancy are comprehend in decidualisation, placentation, steroidogenesis, angiogenesis, and suppression of maternal immune response. Prostaglandin downregulate T cells and NK cells IL-2 receptors and upregulate secretion of IL-10 and receptors for IL-10. Indomethacin treatment of allopregnant mice in early pregnancy may cause resorption 90% of fetuses. This mechanism of fetal resorption includes activation of decidual NK cells and trophoblast cells lysis (41,42).

Domination of Th₂ type immunity very efficiently mediated in fetoplacental unit rejection and pregnancy loss. Athwart, predomination of Th₁ type immunity prevent fetoplacental allograft rejection, contribute fetal survival and may protect endangered pregnancy. In some models, successful therapy of endangered pregnancy is established on usage of Th₂ or Th₃ cytokines (35).

Many trophoblast proteins like oncofetal antigens, cancer/testis antigens, progesteron-induced blocking factor, TJ6, and PSG-18 proteins, also have very important role in feto-maternal relationship. Every of them have immunosuppressive effects or immunomodulatory effects on maternal immune system during pregnancy (43–47).

Another mechanisms for the escape of fetoplacental unit from maternal immune response are mediated by fas/fas-ligand interactions. Trophoblast cells express fas-ligands and may cause apoptosis of fas+ decidual immunocompetent cells. These embryo-protective mechanisms are particularly important for stage of implantation and early trophoblast invasion (48,49). Athwart, some of factors operate like antiapoptotic agents. IL-10, LIF, estradiol, and related steroid hormones may be promoters of antiapoptotic effects of Th₂ cells in pregnancy (38,50–52).

Altered expression of MHC and/or placental antigens by trophoblast cells (absence of expression of MHC antigens and expression of nonclassical MHC antigens like HLA-G)

One such mechanism may be that placental syncytiotrophoblast at the maternal-fetal interface does not express the classic MHC class I and class II molecules except for HLA-C, HLA-E, and HLA-G (53). The precise function(s) of these molecules is unknown, although one function of HLA-G may be to downregulate NK activity within the pregnant uterus (54). Poorly trophoblast HLA-G expression downregulate decidual production of TGF- β ₂, upregulate NK activity and lead in pregnancy loss. Transgenic cell line LCL721.221HLA₀ is very susceptible on NK cell activity, but reconstruction of this transgenic cell line with genes for HLA-G and HLA-A₂ expression, restore significant resistance on NK cell activity (55). In

pregnancy, trophoblast expression of HLA-G increases decidual production of IL-3, IL-10, and IL-1 β and decrease production of TNF- α (53,54). Christiansen et al. (56) demonstrated that maternal HLA-DR alleles DR1/Br and -DR3 or closely linked genes seem to predispose to pregnancy losses in RSA (Recurrent Spontaneous Abortion) patients and their first-degree relatives. Endovascular cytotrophoblast from abnormal pregnancies including preeclampsia and RSA syndrome reacts with anti-HLA-DR and anti-ICAM-1 antibodies (57). Furthermore, attributes of endangered pregnancy are increased decidual NK activity and decidual production of Th₁ cytokines.

Altered expression of adhesion or accessory molecules by trophoblast and/or dendritic cells

Trophoblast cells do not express costimulatory molecules. At the same time, most of decidual immunocompetent cells express very low levels of costimulatory molecules like CD80, CD86, and CD40. Rather, early decidual cells express HLA-DR and CD86 but term decidual cells did not express these antigens (58). Decidual dendritic cells in RSA patients express high level of costimulatory molecules. Success immunotherapy of RSA based on immunization with paternal white blood cells downregulate decidual mononuclear cells expression of CD80 antigens, and downregulate decidual production of Th₁ cytokines (59).

These are only parts of mechanisms, which participate in tolerance of malignant tumor or foreign tissue like trophoblast.

TUMORS, MAMMALS, AND NONMAMMALIANS CLASSES OF VERTEBRATES

Animals like fishes, amphibians, reptiles, and birds go get malignant tumors, but their incidence are significantly lower than in mammals. Efron et al. instigate rate of neoplasia in wild mammals, birds, reptiles, and amphibians. Neoplasia was present at necropsy in 2.75% of 3127 mammals, 1.89% of 5957 birds, 2.19% of 1233 reptiles, and 0% in 198 necropsies of amphibians. Most frequently malignant tumors in birds and reptiles are viruses induced sarcoma. Athwart, most frequently malignant tumors in mammals are cancers with different etiology (60,61). Amphibians also have very low incidence of spontaneous malignant tumors (60–62). Laurens et al. instigate that spontaneous tumors may develop in inbred and isogenic strains of *Xenopus laevis*, the South African clawed toad, they are extremely rare in wild-type populations of all amphibians (62). Cartilaginous fishes like sharks and their relatives, recently no have propensity for malignant tumors, while bonefishes

shows higher incidence of malignant tumors than sharks, but still much lower than mammals, reptiles and birds (63).

Although comparative data for incidence of malignant tumors in vertebrates are hardly available, opinion about ascending incidence of malignant tumors on vertebrate's evolution scale is real.

There are several possible factors for low incidence of malignant tumors in nonmammalian vertebrates in comparison with mammals:

- (a) Primitive immune systems in fishes, amphibians and reptiles are most effective than mature mammalian immune systems. Anti-tumor immunity in these vertebrates is mostly rested on innate immune system, but primary error in mammalian anti-tumor immunity is in communication between innate and adaptive immune system (64).
- (b) Nonmammalian vertebrates (except birds) very slowly reject skin allograft because they have not MHC antigens (65). Poor maturity or absence of MHC molecules in nonmammalian vertebrates (except birds) qualifies substantially different anti-tumor immune response, which is not established on MHC molecules. Mammalian immune response is closely associated with MHC antigens, consequently absence of MHC antigens on tumor cells leads in tumor escape (23).
- (c) Primitive immune systems and differences in MHC systems in nonmammalian vertebrates enable otherwise anti-tumor cytokine network than mammals. Cytokines like IL-10 and TGF- β are unknown in fish and amphibians, but TGF- β is evidenced in reptiles and birds (66,67).
- (d) Mammalian immune system may be tolerating cancer cells because they are very similarly with trophoblast cells.
- (e) Malignant cells in fishes, amphibians, reptiles and birds are most susceptible on apoptosis than mammalian malignant cells (62).
- (f) High resistance on cancerogen induced genetically changes is evidenced in some experiments with lower vertebrates. Thereby arise the opinion that DNK from lower vertebrates shown high resistance on cancerogenesis (62,63).

Instigated and other factors, probably contributed significant anti-tumor immunity failure in mammals.

EXPERIMENTAL MODELS AND INTEREST FOR ANTI-TUMOR IMMUNOTHERAPY

If mechanisms of anti-tumor immunity in mammals are similar or same with mechanisms of immunoregulation in pregnancy, then mechanisms of anti-tumor immunity

in nonmammalian vertebrates may be very useful for immunotherapeutic procedures.

Cytokine network differences of nonmammalian immune response on trophoblast cells or embryonic cells, mammals or nonmammalian tumor cells may indicate which of cytokine network models are most efficient in anti-tumor immune response.

Nonmammalian cytokine may be utilized as adjuvant in anti-tumor immunotherapy and/or anti-tumor vaccination.

Supernatants of *in vitro* (membrane sundered or not) cells culture of immunocompetent cells of nonmammalian vertebrates and trophoblast cells or embryonic cells, mammals or nonmammalian tumor cells may contain anti-tumor factors and/or adjuvant.

Immunocompetent cells from nonmammalian vertebrates derived after *in vivo* or *in vitro* immunization with trophoblast cells or embryonic cells, mammals or nonmammalian tumor cells, may be transplanted into malignant tumor bearers.

TIL from mammalian tumors may be stimulated with nonmammalian cytokines and then returned in circulation of tumor bearers and/or tumor vicinity.

TIL from mammalian tumors may be incubated (membrane sundered or not) in culture of nonmammalian immunocompetent cells and trophoblast cells or mammals or nonmammalian tumor cells and then returned in tumor bearers and/or tumor vicinity.

Stimulated mammalian TIL and/or nonmammalian immunocompetent cells and/or cytokines may be transported into tumor vicinity by liposome.

CONCLUSION

Questions about origin of anti-tumor immunity failure in mammals are still open. Possibility that mammals refunded price for own manner of reproduction in high incidence of malignant tumors is real. Mechanisms of anti-tumor immunity in nonmammalian classes of vertebrates are substantial different than in mammals. Nevertheless, we do not know exactly why nonmammalian vertebrates have below incidence of malignant tumors than mammals. If we get to know mechanisms of anti-tumor and anti-trophoblast immunity in nonmammalian vertebrates, we will be able to design a new anti-tumor immunotherapeutic protocols.

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