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Failure of blood–thymus barrier as a mechanism of tumor and trophoblast escape

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Summary A major process through which the immune system becomes tolerant to self-proteins involves the deletion of self-reactive clones in the thymus, but clonal deletion is not single mechanisms of thymic tolerance. There is now much evidence that intrathymic antigen expression results in anergy induction of T helper type-1 (Th1) clones in the periphery. Blood–thymus barrier is most important structure for prevention of unwanted penetration of antigens into the thymus. Impermeability of the barrier restrain induction of acquired thymic tolerance on unwanted antigens like microbes and tumor cells. Nevertheless, one of most important mechanism of tumor and trophoblast escape is in anergy of Th1 cells and in Th2 cells domination. Many mechanisms are included in disarrangement of Th1/Th2 balance in pregnancy and tumor bearers, but one of possibility is in failure of blood–thymus barrier. Possible consequences of blood–thymus barrier failure are trophoblast-specific or tumor-specific antigens penetrate into the thymus, deletion or anergy of antigen-specific clones and acquired thymic tolerance induction. Blood–thymus barrier is variable structure in anatomical and functional sense so that in certain condition foreign antigens probably can permeate across the barrier. Probability that some factors like hormones, cytokines, prostaglandine and neuromediators can affect blood–thymus barrier permeability and contribute in mechanisms of trophoblast and tumor escape is real but relatively unexplored.

BASIC MECHANISMS OF THYMIC TOLERANCE

Immunologic self-tolerance can occur partly through clonal deletion. Thymic tolerance induction by clonal deletion is achieved in T cells during they maturation in the thymus. There are two critical events in the establishment of a functionally effective yet non-autoreactive T cells pool: positive selection of T cells with a TCR capable of recognizing complex peptide–MHC and negative selection of T cells (1,2). In positive selection only those cell whose recognizes a peptide–MHC molecule are selected for survival. Those cells whose receptors are not MHC restricted do not interact with thymic epithelial cells and consequently do not receive the protective signal, thus leading to their death within 3–4 days via apoptosis (2–4). Negative selection involves the population of MHC-restricted reactive thymocytes that survive positive selection comprises of some cells with low-affinity receptors for self-peptide presented within self-peptide–MHC molecules complexes and other cells with high-affinity receptors. During negative selection, dendritic cells and macrophages bearing class I and class II MHC molecules are thought to interact with thymocytes bearing high-affinity receptors for self-peptide–MHC complex, or self MHC molecules alone. The nature of the interaction is unknown, but cells with receptors for self-peptide–MHC complex or MHC molecules alone undergo death by apoptosis (3,5).

Another mechanism of immunologic self-tolerance is based on mechanisms called 'clonal anergy'. The remaining non-deleted, autoreactive thymocytes are temporarily unable to mediate immune reactions and

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functionally silent. Some authors are found that low-level antigen intrathymic expression results in anergic thymocytes, which also show a reduction of Th1 activity with no decrease in Th2 activity (*6*). Although thymic macrophages have been implicated in deleting autoreactive thymocytes, their also presents antigens to an IL-4-producing Th2 cells after IFN-γ treatment as evidence by T cell proliferation and the release of IL-3 and IL-4. However, these thymic macrophages are inefficient at stimulating IL-2 producing Th1 clone (*7*). Antonia et al. (*6*) propose that the loss of Th1 activity as a consequence of the thymic epithelium being encountered by tissue-specific proteins results in the functional tolerization of CTL in vivo, despite the fact that CTL are fully functional in vitro. Thymic expression of peripheral proteins may therefore be an additional way in which tolerance to peripheral proteins can be achieved (*6*). Experiments with acquired thymic tolerance induction to foreign MHC molecules in neonatal mice also shown presence of Th1 anergy as possible mechanisms of the thymic tolerance. In mixed lymphocyte reaction, spleen cells from normal mice proliferated in response to 'tolerogen', generated cytotoxic cells and produced IL-2 and IFN-γ but no IL-4 or IL-5. In contrast, although spleen cells from tolerant mice proliferated and produced IL-2, they failed to generate cytotoxic cells or produce IFN-γ but produced large amounts of IL-4 and IL-5. Overall the results point to a profound switch in peripheral tolerogen-specific responses from a Th1 biased response in normal mice to a Th2 biased response in tolerant mice (8).

BLOOD–THYMUS BARRIER PERMEABILITY AND THYMIC TOLERANCE

Studies of blood–thymus barrier permeability in late fetal and adult stage showed that cortical thymocytes are protected from circulating macromolecules. The adult blood–thymus barrier consist capillary endothelial cells, basal lamina of endothelium, perivascular space, basal lamina of the epithelial-reticular cells and the epithelial-reticular cells. In contrast to cortical thymocytes, the medullar thymocytes are unprotected from circulating macromolecules. Blood vessels in the thymic cortex were not permeable to macromolecules, but the post-capillary venules of the thymic medulla permitted the macromolecules to leak along the clefts between migrating lymphocytes and endothelial cells. The macromolecules, which crossed medullary venule walls, had limited distribution in the thymic parenchyma because macrophages in the perivascular space ingest and retain much of the leaked macromolecules (*9*–11).

Most important function of blood–thymus barrier in postnatal life is prevention of foreign antigens penetration into the thymus. Intrathymic inoculation of the antigen induces acquired and specific immune tolerance to the antigen. Model of acquired thymic tolerance in adulthood by intrathymic antigen inoculation is utilized for tolerance induction in transplantation immunology (12,13). Chen et al. (13,14) demonstrate that antigen intrathymic injection results in apoptotic cell death of both CD4+ and CD8+ the thymocytes. Furthermore, thymectomy after intrathymic injection abrogates the effect of acquired thymic tolerance and restores antigen-dependent clonal expansion in vivo. Moreover, these authors concludes that intrathymic injection of antigen induces Th1 cell unresponsiveness and prevents the peripheral expansion of antigen-specific CD4+ T cells in vivo (13,14). Many authors used intrathymic inoculation of antigens for prevention some autoimmune disease. Intrathymic injection of guinea pig myelin basic protein without otherwise compromising the peripheral lymphocyte pool in adult rats dramatically inhibits onset of experimental allergic encephalomyelitis caused by the usual peripheral inoculation with in complete Freund's adjuvant (15,16). Other authors used inoculation of thymic epithelial cells, MHC molecules, lymphocytes or other MHC expressing cells of a donor, into the thymus of a recipient of the allogeneic graft (17,18). Adult animals, recipients of donors cells or MHC molecules and donors allogeneic the graft, significantly slower reject the graft in comparison with control animals. These authors instigate that intrathymic inoculation of MHC expressing cells from donor to recipient induce acquired and specific thymic tolerance to allogeneic the graft. Intrathymic injection of an immunodominant peptide induces acquired thymic tolerance and suggests an indirect pathway of allorecognition in the thymus (17,18). Intrathymic inoculated genetically engineered dendritic cells expressing donor MHC class I or II molecules or a peptide analogue might have therapeutic potential in the induction of transplant tolerance and in the treatment of autoimmune diseases (18). Thymic tolerance was given with intrathymic injection of UV-B-irradiated spleen cells or purified resting allogeneic T cells, but not resting B cells, dendritic cells, or macrophages. Thymectomy performed 7 days after intrathymic injection led to graft rejection strongly suggests that the early phase of induction of donor-specific tolerance is dependent on the presence of donor alloantigens in the host thymus (19).

There is now evidence that some factors can reverse permeability of blood–thymus barrier and contribute to intrathymic penetration of foreign antigens. Sex steroids like estradiol, progesteron and glucocorticoids can impair blood–thymus barrier. For now, we know for thymic involutive factors like stress, ACTH and steroid hormones as factors, which can unclose blood–thymus barrier for foreign antigens (10,20,21).

TH1/TH2 DISARRANGEMENT IN MECHANISMS OF TUMOR AND TROPHOBLAST ESCAPE

Th1 and Th2 cells, and their ratio play important immunoregulatory roles. Largely Th2 immunity is ineffective in anti-tumor response. Athwart, Th1 immunity and CTL or NK cells after stimulation by Th1 cytokines (IL-2, IL-12, IFN-γ, and TNF-α) shows very efficient anti-tumor effects. Some recent studies have demonstrated that an elevated level of Th2 cytokines, such as IL-4, IL-6, IL-10 and TGF-β, contributes to the ability of cancer cells to escape immunosurveillance. High levels of IL-6, IL-10 or TGF-β have been found in serum of patients with advanced tumors like lung cancer, melanoma, breast cancer, pancreatic carcinoma or with disseminated the metastases. Tumor regression is very often associated with downregulation of IL-10 and other Th2 cytokines production (22–26). Kim et al. (27) showed that intralesional treatment with IFN-α induced tumor regression, associated with downregulation of IL-10 mRNA. In some models, successful immunotherapy of established tumor is associated with change in the balance of T cell subset from Th2 to Th1 phenotype (28). Today we know for several factors that participate in disarrangement of Th1/Th2 balance in tumor bearers like unusual HLA-G expression, poorly MHC-molecules expression, good expression of Fas-ligands, absence of co-stimulatory molecules expression, increased prostaglandine secretion, increased steroid hormones secretion, biased present cytokine network, immunomodulatory oncofetal antigens and cancer-testis antigens secretion, and the other factors.

Spontaneous abortion mediated by immune mechanisms is common complication of pregnancy. Domination of Th2 type immunity successful prevents fetoplacental allograft rejection, contributes to the fetal survival and may protect endangered pregnancy. In some models, successful therapy of endangered pregnancy is established on usage of Th2 or Th3 cytokines (29,30). Cytokines like TGF-β, IL-10 and IL-6 have very important role in mechanisms of trophoblast protection from maternal immune response. These cytokines strongly inhibits secretion of cytokines like IL-1β, IL-2, TNF-α and TNF-β, significantly decelerates rejection of any allograft and promote Th2 immune response (29,31). Domination of Th1 type immunity very efficiently mediated in fetoplacental unit rejection and pregnancy loss. In patients with recurrent spontaneous abortion (RSA), level of decidual production of IL-1, IL-2, IL-12, IL-18, TNF-α and IFN-γ is significantly enhanced than in healthy pregnant women. Activity of decidual NK cells after spontaneous abortion is also increased in regard activity of decidual NK cells in healthy women (29,30). Many factors are associated with domination of Th2 immunity in normal pregnancy like unusual HLA-G expression, poorly MHC-molecules expression, good expression of Fas-ligand, absence of co-stimulatory molecules expression, increased prostaglandine secretion, increased steroid and sex hormones secretion, biased present cytokine network, secretion of immunomodulatory oncofetal antigens, PSG-18 and TJ-6 proteins, cancer-testis antigens, and the other factors.

TUMORS, PREGNANCY AND THE THYMUS

Characteristics of both pregnancy and tumor development the conditions are domination of Th2 immunity, lymphocyte clonal anergy, and immunotolerance of proliferative tissue. Although tumor and trophoblast immunity failure are mostly result of local events in tumor's and decidual-trophoblast microenvironments, central thymic tolerance in these processes are not excluded (27,29).

Pregnancy as well as tumors is associated with an involution of the thymus accompanied by a massive depletion of the cortical region and alteration in the distribution of thymocytes, with a decrease in CD4+CD8+, CD4+CD8− and CD4−CD8+ thymocytes. However, CD4−CD8− population shows an increase, suggesting impairment in thymocytes differentiation at an early T cell maturation stage (32,33). Adkins et al. (34) investigated three possible mechanisms leading to this thymic atrophy: (1) increased apoptosis, (2) decreased proliferation, and (3) disruption of normal thymic maturation. Their findings suggest that the thymic hypocellularity seen in mammary tumor bearers is not due to a decreased level of proliferation, but rather, to an arrest at an early stage of thymic differentiation along with a moderate increase in apoptosis (34). Enhanced levels of glucocorticoids are known to produce similar effects on the thymus, but adrenalectomy of mice followed by tumor implantation did not result in reversal of the thymic atrophy. Furthermore, a study of serum corticosterone levels in tumor bearers indicated no significant changes during tumor development. Because no major changes were observed in tumor bearers, either at their capacity to repopulate the thymus or at the patterns of subsequent redistribution of thymocytes, it was postulated that the thymic atrophy might be caused by a direct or indirect effect of the tumor or tumor-associated factor(s). Intrathymic injections of tumor cells into young normal recipient mice resulted in a significant reduction of the thymus weight and cellularity (35). The mechanism of thymic involution during tumor growth probably is related to inhibition of thymocytes' proliferation, impaired differentiation and enhanced intrathymic death caused by cytokine release from the non-lymphoid thymic population (34).

The thymus changes dramatically during pregnancy. It shrinks in size, and the cortex is extensively reduced from midpregnancy onwards. Other changes associated with pregnancy involves the medullary epithelial cells that undergo an increased level of mitosis. Their greater numbers surround accumulations of lymphocytes to form the characteristic medullary epithelial rings. Cell movement through blood vessel walls was clearly observed in midpregnancy, but not at other times (33). In vitro, trophoblast cells inhibited almost completely the proliferative response of thymocytes whether or not combined with the thymic stroma. Decidual cells were also found to have an inhibitory effect on thymocytes proliferation while their combination with the thymic stroma decreased the thymocytes proliferation rate almost completely (36). Whilst the cortex shrinks, the medulla enlarges and rearranges to create a microenvironment containing increased numbers of mature thymocytes. Clarke and Kendall (37) suggests that these recently derived T cells may contribute to the unique populations of cells with suppressive function that appear during pregnancy, and thereby contribute to the immune suppression of the mother to paternal and fetal antigens. In addition, the pregnancy-associated cortical involution of the thymus may reflect the deletion of clones with potential reactivity to paternal and/or fetal antigens (37).

FAILURE OF BLOOD–THYMUS BARRIER AS A MECHANISM OF TUMOR AND TROPHOBLAST ESCAPE

Today we know that involutive factors like stress, ACTH, sex hormones, glucocorticoids, simultaneously increase blood–thymus barrier permeability. Additionally, Lauritzsen et al. (38) are found that circulating myeloma antigens is processed and presented by thymic antigen presenting cells, and induces deletion of the antigen-specific thymocytes. Deletion of tumor-specific thymocytes may represent a tumor escape mechanism in patients with cancers that secrete or shed tumor antigens (38). However, there is no much evidence about influence of cytokines and other tumor and trophoblast escape factors on the blood–thymus barrier permeability. For now, we know that some of pro-inflammatory cytokines induce thymic involution. In same time, some of anti-inflammatory cytokines and growth factors are strong anti-involutive factors. Cytokines like IFN-β is associated with massive apoptotic death of thymocytes and thymic involution. In this process, CD4+CD8+ T cells decreased significantly, whereas there were relative increases in CD4−CD8−, CD4+CD8−, and CD4−CD8+ T cells pool. High doses of IL-2 also cause the thymic involution (39). In contrast to TNF-β and IL-2, cytokines like IL-10 and IGF-I is strong anti-apoptotic and anti-involutive factor of the thymus (40).

Current cognition maintained hypothesis that involutive factors increase the blood–thymus barrier permeability as well as hypothesis that increased permeability of the barrier might be a factor of central and peripheral thymic tolerance. Many cytokines, oncofetal antigens, soluble receptors, prostaglandine, progesteron, estrogens, trophoblast factors and other factors, which characterized pregnancy and tumor's microenvironment, probably can impair permeability of blood–thymus barrier. As results of these events, circulating tumor-specific, trophoblast-specific or paternal antigens can permeate blood–thymus barrier and induces acquired thymic tolerance. In view of the acquired thymic tolerance as phenomenon of selection and anergy of thymocytes, failure of blood–thymus barrier as reason of the thymic tolerance in mechanisms of tumor and trophoblast escape is particularly possibly. Mechanism of specific thymic selection in tumor bearers is already described (38), but this mechanism in pregnancy is yet unexplored. Mechanism of Th1 lymphocyte anergy in pregnancy and in tumor bearers as result of blood–thymus barrier failure also is unexplored.

CONCLUSION

If blood–thymus barrier permeability is associated with central thymic tolerance and Th1 anergy in mechanisms of tumor and trophoblast escape, disclosure of factors that can affect the barrier may contribute detection of new possibility in anti-tumor immunotherapy and therapy of immunopathologic pregnancy.

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