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Crossroads of extrathymic lymphocytes maturation pathways

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Summary The majority of T cells located in peripheral lymphoid organs are dependents on the thymus for regular differentiation and function. Only a minority of T lymphocytes are thymus-independent. These cells pass by extrathymic maturation processes and become mature T lymphocytes. Some data suggest that mechanism of extrathymic lymphocytes maturation (eTLM) includes migration, proliferation, differentiation and selection of lymphocytes as well as thymic pathway. With aging and progression of thymic involution or in accidental thymic involution, pathway of eTLM derives emphasis. T cells from extrathymic pathway probably can polarize action of thymic-dependent T cells or participate in immune reaction in antigen-destructive or antigen-protective manners. Consequently, extrathymic pathways can be a source of self-reactive T cells or cells which participate in mechanisms of trophoblast or tumor escape. Results of eTLM probably are not presets, already depend upon many factors and microenvironmental snapshots. Factors like cytokines, prostaglandine, microbes, MHC molecules, hormones, Fas ligand, heat shock proteins, phenotypes of dendritic cells and APCs, probably can be polarizing courses of eTLM pathway. Definitive to the course of extrathymic-derived cells action, presumably is resultant of microenvironmental relations and interactions of foregoing factors. Hypothesis that microbes, especially viruses, can be promoters of extrathymic (self)antigen-reactive lymphocytes maturation is real as well as hypothesis that extrathymic lymphocytes selection and products of selected lymphocytes can be included in mechanisms of tumor, trophoblast and transplant rejection or escape.

THYMIC AND EXTRATHYMIC LYMPHOCYTE MATURATION

Intrathymic lymphocyte maturation includes several closely related processes: migration, proliferation, differentiation and selection of thymocytes. The developing T cells within the thymus initially populate the outer cortex, where they undergo population growth, rearrangement of TCR genes and surface expression of CD3, TCR, CD4 and CD8 molecules. As thymic lymphocytes mature, they migrate from cortex to medulla. During this migration, lymphocytes go through three main developmental stages defined based on CD4 and CD8 expression. In the earliest maturation stage, the cortical thymocytes are $TCR\alpha\beta^+$ or $TCR\gamma\delta^+CD4^-CD8^-$ double negative (DN) cells. Afterward, they became $TCR\alpha\beta^+CD4^+CD8^+$ double positive (DP) cells. $TCR\gamma\delta^+$ lymphocytes are DN in stages of full maturity, while $TCR\alpha\beta^+$ lymphocytes are DN only in earliest stages of intrathymic development. Finally, mature medullar thymocytes are $TCR\alpha\beta^+CD4^+CD8^-$ or $TCR\alpha\beta^+CD4^-CD8^+$ single positive (SG) cells. Adult thymus contains about 5% DN, 80% DP 12% $TCR\alpha\beta^+CD4^+CD8^-$ and 3% $TCR\alpha\beta^+CD4^-CD8^+$ SG lymphocytes. During these intrathymic transformations lymphocytes pass through positive and negative selection. Self-reactive thymocytes die by apoptotic mechanism, but remainder self-tolerant thymocytes become mature lymphocytes and soon leave the thymus (1–3).

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The thymus is the major site of maturation of both helper T (Th) and cytotoxic cells (CTLs). If the thymus is removed from a neonatal mouse, this animal does not develop a normal T cell repertoire and remains in T cells throughout its life. The congenital absence of the thymus in humans (Di George syndrome) or in the ''nude'' mouse strain is characterized by low mature T cells in peripheral lymphoid organs and circulation, and deficiencies in T cell mediated immunity (4,5). However, the fact that some phenotypic mature and functional T cells do exist in athymic individuals or animals suggests conclusion that extrathymic sites of T cell maturation may exist. It may be that the remnant of the involuted thymus is adequate for some T cell maturation or that other tissues can assume the role of the thymus. Today we know that some T cells called extrathymic T cells have been found to become mature lymphocytes in extrathymic conditions without support by the thymus.

Tamauchi et al. (*6*) have demonstrated that TCR$\alpha\beta^+$ DP thymocytes injected in athymic mice can develop into TCR$\alpha\beta^+$CD4$^+$CD8$^-$ and TCR$\alpha\beta^+$CD4$^-$CD8$^+$ SG cells in extrathymic tissues. Same authors have experimental results that liver cells can influence lymphocyte maturation in athymic nude mice (*6*). Moreover, RAG1, RAG2 and pre-T cell receptor α chain expression as markers of lymphocyte immaturity are discovered in adult human hepatic T cells (*7*). These results suggest that pre-T cells are trafficking from the bone marrow or the thymus to other tissues to continue differentiation and selection in the context of an appropriate cellular and molecular environment. The presence of immature populations of T cells in the adult liver and high levels of RAG expression suggests that the adult liver provides such an environment for extrathymic T cell maturation. Except liver, there are many evidences that extrathymic lymphocytes maturation (eTLM) is possible in other tissues like epithelial tissue and peripheral lymphoid organs, for example Peyer's patches (8–10).

Thymic epithelial cells, dendritic cells and macrophages play an important role in processes of thymic maturation, including positive and negative selection. At the same time, there are data that similar cells are included in extrathymic lymphocyte selection (*6*,*9*,11). Kadena et al. (12) have found that the TCR$\alpha\beta^+$ DN T cells develop through a unique extrathymic pathway through bacterial induced activation and accumulation of peritoneal macrophages (12). Signal for activation of eTLM probably is TNF-β, while same signal can be inhibitor of thymic maturation pathway (12,13). Moreover, there are data that eTLM process includes selection of self-reactive clones. Extrathymic selection process probably may go according to apoptotic death of self-reactive clones, but proliferation and full maturity of the clones is also possible. In vitro studies have demonstrated antigen-specific induction of apoptosis in TCR$\alpha\beta^+$ DP thymocytes cultured in suspension, by thymic as well as splenic APCs. Thus, the recognition of antigen by TCR$\alpha\beta^+$ DP thymocytes may lead to clonal deletion, which is not limited by the antigen-presenting ability of the thymic stroma (14). Because population of immature extrathymic T cells comprise both TCR$\alpha\beta^+$ cells and TCR$\gamma\delta^+$ cells in different maturation stages, some authors have evidences about phenomenon of TCR gene rearrangement in extrathymic lymphocytes (15–18). Sites of eTLM contain DN self-reactive oligoclones and constitutively express the IL-2 receptor β-chain (15). Borenstein et al. (*19*) showed that neonatal MHC class I tolerance in the adult is associated with low-level hematopoietic chimerism and extrathymic deletion of alloreactive SG CD8$^+$ T cells. These data indicate that the process of (self)reactive clones negative selection or (self)antigen-reactive clones production like extrathymic phenomenon is possible (*19*).

SITES OF eTLM

Except the major sites of extrathymic T cells maturation like the intestine and liver, it is well known that pregnant uterus, decidua, microenvironments of malignant tissues, allogeneic transplants, autoimmune and infective focuses also can be real sites of eTLM. Because sites of eTLM in more cases are temporary and poorly anatomical defined, any similarity and/or differences between thymic and eTLM pathways are hardly for demonstrations. However, extrathymic pathway of lymphocyte maturation probably plays a pivotal role in immune mechanisms of autoimmune diseases, malignancies, pregnancy and response to microbes. At least, mechanism of eTLM can be one of the universal mechanisms of immune reactions control.

Liver and intestine contain large proportion of TCR$\gamma\delta^+$ cells as well as TCR$\alpha\beta^+$ cells, also contain DN cells and self-reactive oligoclones. These cells constitutively express the IL-2 receptor β-chain, and have a $\alpha\alpha$ homodimer of CD8$^+$, while intraepithelial lymphocytes TCR$\alpha\beta^+$ cells were mainly IL-2R β- and contained both DP cells and SG cells. CD4$^+$ cells were more predominant than CD8$^+$ cells in the liver, while CD8$^+$ cells were persuasively predominant in the intestine (5,15). A specific cell population, the DP cells, normally present in liver and the thymus, but in liver the population shows significant increase in number during viral hepatitis. At the same time, no significant changes in T cell subpopulations were detected in the spleen. These observations suggest that viral infection could induce an early in situ stimulation of resident hepatic T cells, despite a peripheral immunodeficiency in the thymus and spleen (20). Pregnancy is the condition of Th2 cells domination and

proliferative trophoblast toleration. Shift in domination of Th2 cells subpopulation happens in second phase of menstrual cycle and in early pregnancy. These events are results of immunosuppressive and immunomodulatory factors activity in pregnancy (21). However, some authors are of the opinion that shift in endometrial and decidual lymphocyte subpopulations are the result of eTLM (22).

In accordance with previous data, Lee et al. (23) have detected that population of tumor infiltrating lymphocytes contains about 15% of DP immature lymphocytes (23). These data maintained an opinion about tumor tissues like microenvironment of intensive eTLM.

Although DP T cells are rarely present in human peripheral blood, the relative percentage of this lymphocyte population can increase spontaneously in healthy individuals and in persons suffering from certain disease conditions. These cells can also be found among those T cells infiltrating arthritic joints, rejected kidney grafts and certain tumors (24). Experiments with neonatally thymectomized showed that the mice fall victim to such autoimmune diseases as gastritis and pancreatitis with aging. Self-reactive T cell clones can be consistently generated in the absence of the thymus, and these clones via the extrathymic pathways and an alternative pathway probably are responsible for autoimmune disease induction (25).

After intraperitoneal inoculation with *Listeria monocytogenes*, TCR$\gamma\delta^+$ cells appear in the peritoneal cavity preceding the appearance of TCR$\alpha\beta^+$ cells. Such TCR$\gamma\delta^+$ cells predominantly express T cell receptor TCRγ1/Vδ6, develop through an extrathymic pathway, and contribute to host defense against the bacteria (26). Results from other authors suggested that Th2-associated responses also can be induced by nematode infection in rats through the extrathymic recruitment and proliferation of $CD4^+CD8^-$ SG TCR$\alpha\beta^+$ T cells (27). Mouse hepatitis induced with virus type 3 is an excellent model for the study of thymic and extrathymic T cell subpopulation disorders induced during viral hepatitis. It was recently reported that, in addition to the intrathymic T-cell differentiation pathway, an extrathymic differentiation pathway of TCR$\alpha\beta^+$ T lymphocytes exists in the liver, and becomes important under pathological situations such as autoimmune diseases, malignancies or hepatic bacterial infections (20).

CROSSROADS OF eTLM pathways

Some authors propose the possibility that switching of the immune system from the thymus to the alternative eTLM sites might be regulated by the autonomic nervous system as well as by cytokines. Extrathymic T cells are very few in number at any extrathymic sites in adolescence, but they increase in number as a function of aging (5,15). This process probably is parallel with progression of thymic involution. Moreover, acute thymic atrophy always accompanies activation of eTLM. Therefore, reciprocal regulation between extrathymic T cells and thymus-derived T cells might be present.

Cytokines like IL-7 have very important role in extrathymic T cell development. Under influence of this cytokine, human hematopoietic stem cells can develop into mature TCR$\alpha\beta^+$ lymphocytes and immature progenitors in the bone marrow of athymic mice (28). Extrathymic TCR$\alpha\beta^+$ can be detected, but not TCR$\gamma\delta^+$, in IL-7 gene-deleted animals, suggesting that alternative cytokines may be involved in eTLM (29,30). Porter and Malek (31) have discovered that eTLM is regulated by cytokines like IL-2, IL-7 and IL-15, but IL-2 is most important for thymic pathway according such as IL-15, most important for eTLM (31,32).

Extrathymic dendritic cells and APCs can be associated with eTLM and clonal selection as well as thymic dendritic cells and APCs. There is evidence about antigen-specific induction of apoptosis in $CD4^+8^+$ thymocytes cultured in suspension, by thymic as well as splenic APCs. Thus, the recognition of antigen by $CD4^+8^+$ thymocytes may lead to deletion, suggesting that this is the central mechanism of tolerance induction, which is not limited by the antigen-presenting ability of the thymic stroma (33).

Steroid hormones probably can be activators of eTLM, at the same time; extrathymic matured lymphocytes are more resistant to steroids activity in relation to thymic lymphocytes (34).

Data about activation of eTLM by stress in adolescence indicate relationship between stress, steroids, thymic atrophy and eTLM. Most likely, majority of conditions with thymic atrophy like pregnancy and malignant tumors are accompanied with eTLM activation. Direction of eTLM probably depends upon many factors and microenvironmental status. To that effect, some of the factors that might be polarizing eTLM pathway probably are microbes, costimulatory molecules, Fas ligand, prostaglandine, sex hormones, heat shock proteins and expression of MHC molecules. After activation of eTLM center, microenvironmental conditions probably can determinate reactivity of extrathymic-derived lymphocytes, according to (self)antigen-reactive or antigen-protective way. For example, microbes and immunostimulatory factors like Th1 cytokines probably might be factors of eTLM polarization, according to (self)antigen-reactive manner of immune reaction. Allowing for this model, (self)antigen-reactive manner of eTLM possibly includes positive selection of (self)antigen-reactive thymus-independent lymphocytes, activation of thymus-dependent lymphocytes like CTL

and Th1 cells, NK activity stimulation, suppression and/or negative selection of Th2 and suppressor cells (Figs. 1 and 3).

In the events of microenvironmental domination of Th2 cytokines and other immunosuppressive/immunomodulatory factors, mechanism of eTLM probably includes negative selection of antigen-reactive thymus-independent lymphocytes, suppression of thymus-dependent lymphocytes like CTL and Th1 cells, NK activity suppression, activation and/or positive selection of Th2 antigen-reactive and suppressor cells (Figs. 2 and 3).

Hypothesis about eTLM as phenomenon of in situ lymphocytes maturation that includes mechanisms of clonal selection and changes in composition of lymphocyte subpopulations, may originate some models of immune reactivity in different conditions (Fig. 3).

Based on the described models of eTLM, participation of extrathymic-derived lymphocytes in autoimmunity or tumor, trophoblast and transplant rejections or escape probably is very considerable.

Autoimmunity may be the result of thymic involutive factors activity in coexist with activation of eTLM and microbes, especially viruses. Under these conditions, focuses of eTLM generate self-reactive thymus-independent lymphocytes, belike by positive selection mechanisms. At one time, activation of thymus-dependent CTL and Th1, and NK cells happens. Since pregnancy and malignancy are phenomena of thymus involution, similar processes may be included in mechanisms of miscarriages and tumor rejection.

As distinct from mechanisms of tumor escape or trophoblast escape in successful pregnancy probably are based upon thymic involution, activation of eTLM and interaction with many immunosuppressive/ immunomodulatory factors, but in the absence of microbes. Under these conditions, focuses of eTLM generate suppressor lymphocytes, during thymus-independent antigen-reactive clones die by apoptosis, belike by negative selection mechanisms. Inhibition of thymus-dependent CTL and Th1, and NK cells also happens. In addition, ability that eTLM participate in activation and/or positive selection of Th2 and Th3 cells in mechanisms of *trophoblast and tumor escape* as well existed.

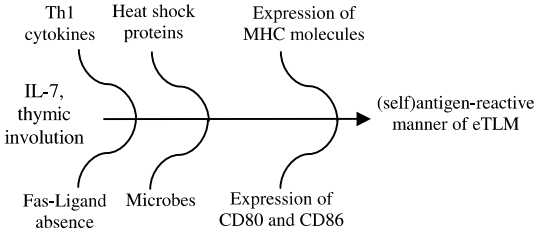

Fig. 1 Model of (self)antigen-reactive manner of eTLM.

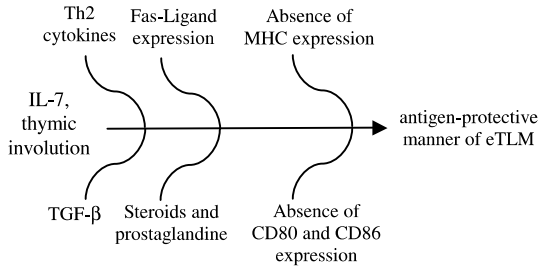

Fig. 2 Model of antigen-protective manner of eTLM.

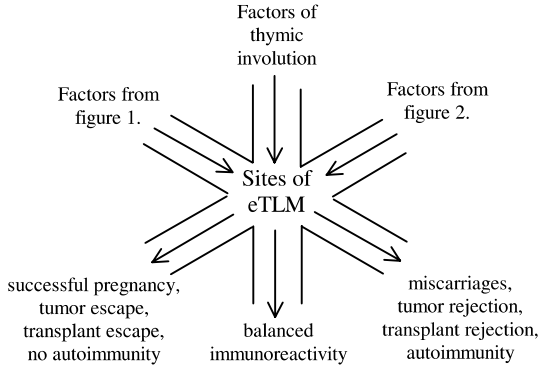

Fig. 3 Crossroads of eTLM pathways.

CONCLUSION

There are now much evidences that thymic involution may be a factor of eTLM activation. Authentic nature of the phenomenon is still undiscovered, but it may be a demand for permanent adaptation of immune reaction control and permanent revision and reselection of (self)antigen-reactive clones. eTLM as phenomenon of in situ lymphocyte maturation, also can be a universal mechanism of immune reaction control. Since mechanisms of eTLM probably are very important, especially after thymic involution, identification of factors that can determinate course of eTLM may be one of the most important problem in the future. Identification of factors that participate in crossroads of extrathymic lymphocyte maturation pathways may be helpful for understanding phenomena like autoimmunity, miscarriages, transplant rejection, trophoblast and tumor escape.

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