ISSN 0006-2979, Biochemistry (Moscow), 2007, Vol. 72, No. 9, pp. 983-988. © Pleiades Publishing, Ltd., 2007.
Original Russian Text © S. V. Shirshev, E. M. Kuklina, A. Yu. Maksimov, O. A. Krapivina, N. S. Parshakova, 2007, published in Biokhimiya, 2007, Vol. 72, No. 9, pp. 1207-1213.
Originally published in Biochemistry (Moscow) On-Line Papers in Press, as Manuscript BM07-132, August 19, 2007.

Extrathymic Rearrangement of αβT-Lymphocyte Antigen Receptor Genes during Pregnancy

S. V. Shirshev, E. M. Kuklina*, A. Yu. Maksimov, O. A. Krapivina, and N. S. Parshakova

Institute of Ecology and Genetics of Microorganisms, Ural Branch of the Russian Academy of Sciences, ul. Goleva 13, 614081 Perm, Russia; fax: (3422) 646-711; E-mail: ibis_07@mail.ru

Received May 4, 2007

Abstract—The existence of αβT-lymphocyte differentiation processes have been demonstrated in mouse peripheral lymphoid organs during pregnancy. Study of pregnant Swiss mice has shown that the development of the second half of gestation is accompanied by expression of RAG-1 recombinase mRNA and unrearranged TCR α-chain (pre-TCRα) preferentially in T-lymphocytes of lymph nodes involved in uterine drainage (para-aortal lymph nodes), and to a lesser extent in other lymph nodes (mainly from axillary lymph nodes). The data suggest that during pregnancy the differentiation of αβT lymphocytes may occur not only in central (thymus) but also in peripheral lymphoid organs.

DOI: 10.1134/S000629790709009X

Key words: αβT-lymphocytes, TCR gene rearrangement, RAG-1, pre-TCRα, pregnancy

According to the traditional concept, maturation and differentiation of αβT-lymphocytes occur mainly in the thymus. During thymic development, T-cell precursors form an antigen specific receptor (TCR) and undergo clonal selection, which eliminates autoreactive clones [1, 2]. Resultant mature T-lymphocytes appearing on the periphery effectively carry out the functions of immune control including recognition and removal of foreign antigens without damage to the organism's own tissues.

Under certain physiological and pathological conditions accompanied by thymus atrophy, alternative extrathymic differentiation pathways become activated; this involves not only γδT-lymphocytes, which traditionally exhibit extrathymic maturation [3, 4], but αβT-cells as well. Such compensatory mechanism operates in stress [5], autoimmune [6, 7] and some infection diseases [8, 9], and also in cases of age-related changes [10]. It is reasonable to suggest that similar mechanism may also function during pregnancy, which is accompanied by significant thymus atrophy: marked decrease of thymus mass [11] and cell number [11, 12] mainly due to cortical atrophy and exhaustion of the population of cortical thymocytes [12]. Involvement of activation of extrathymic development of αβT-lymphocytes in this period is also supported by the following data: administration of the pregnancy associated factors, oncostatin M [13] or high dose of estrogens [14], induces appearance of peripheral αβT-cells with immature phenotype ($CD4^+CD8^+$) [13] or with intermediate level of TCR expression suggesting their extrathymic origin [14].

The goal of the present study was to get experimental evidence for the existence of αβT-lymphocyte differentiation in peripheral organs of mice during pregnancy. Expression of two factors served as a criterion for this differentiation. The first factor is RAG-1 recombinase, the key enzyme in TCR gene rearrangement. Under normal conditions, expression of this enzyme in αβT-cells is limited by the thymic stage of development, and after termination of rearrangement and formation of functional TCR it is irreversibly suppressed [15]. The second factor is unrearranged TCR α-chain (pre-TCRα) expressed by a subpopulation of early thymic precursors lacking fully competent TCRα ($CD4^-CD8^-$) and serving as the marker of immature αβT-cells [16].

Pregnancy is a phenomenon of natural allotransplantation, which requires existence of effective regulatory mechanisms for prevention of possible antifetal immune reactions. Consequently, local immunity has to play the major role in this process. This explains our particular interest in evaluation of putative extrathymic differentiation in regional lymph nodes involved in uterine drainage and others.

Abbreviations: CD) clusters of differentiation (membrane molecules of T-lymphocytes); OsM) oncostatin M; RAG) recombination activating genes; TCR) T-cell receptor; pre-TCRα) unrearranged TCR α-chain.

* To whom correspondence should be addressed.

MATERIALS AND METHODS

Swiss mice and also female CBA/J mice mated with males from various strains were used in experiments. There were several types of mating with BALB/c males (normal allogenic pregnancy), CBA/J males (normal syngenic pregnancy), and DBA/2 males (pregnancy with increased risk of abortion) [17, 18]. Animals were obtained from the Rappolovo Animal Breeding Farm, Russian Academy of Medical Sciences (St. Petersburg). Swiss female mice were investigated on the 5th, 11th, and 17th days of pregnancy, which was detected by a vaginal plug. Pregnancy period in linear animals (9th day) was chosen in accordance with literature data that in CBA/J × DBA/2 females, abortions usually occur on 10-14th day of pregnancy [17, 18].

Thymocytes and fractionated peripheral T-lymphocytes isolated from four types of lymph nodes were used as the research objects: para-aortal nodes (involved in uterine drainage) and also inguinal, mesenterial, and axillary nodes (unrelated to uterine drainage) [18]. In the main series of experiments, B-lymphocytes were removed from cell suspension obtained after homogenization of lymph nodes (for T-lymphocyte fractionation) by means of specific antiserum to mouse immunoglobulins (Medgamal, Russia). (Peripheral B-lymphocytes can rearrange chains of the antigen receptor [19].) In the final series of experiments, αβT-lymphocytes were eliminated from the cell suspension by immunomagnetic fractionation using monoclonal antibodies H57-597 to β-chain of mouse TCR (Caltag, USA). This excluded putative effect of minor subpopulation of γδT-lymphocytes traditionally differentiating outside thymus and also expressing RAG proteins on the periphery [3, 4].

In the isolated cells, expression of mRNAs of recombinase RAG-1, pre-TCRα, and β-actin (positive control for the presence of mRNA in the sample) was analyzed. Obtaining of total RNA, reverse transcription, and amplification were carried out using the kits Trizol RNA Prep 100, GenePak RT Core, and GenePak PCR Core (Isogen Laboratory, Russia), respectively.

The following specific primers and corresponding modes of amplification have been used: for RAG-1, 5′-CATCGAGACAGTCCCTTCC-3′ and 5′-CGATAGAGCCATCCCTTTC-3′ [20] (92°C for 30 sec, 60°C for 30 sec, 72°C for 60 sec (35 cycles)), for pre-TCRα, 5′-ACACTGCTGGTAGATGGAAGG-3′ and 5′-CGAGCAGAAGCAGTTTGAAGAG-3′ [21] (92°C for 30 sec, 52°C for 30 sec, 72°C for 60 sec (38 cycles)), for β-actin, 5′-TGTTACCAACTGGGACGACA-3′ and 5′-TTTGATGTCACGCACGATTT-3′ (chosen by using the program Primer 3: http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) (92°C for 30 sec, 60°C for 30 sec, 72°C for 60 sec (30 cycles)).

Electrophoresis was carried out using the standard method and 1.5% agarose gel (Serva, Germany).

RESULTS AND DISCUSSION

In nonpregnant Swiss mice, mRNA RAG-1 expression has been found only in thymocytes but not in T-lymphocytes isolated from all groups of lymph nodes studied (Fig. 1). This suggests that in the absence of pregnancy differentiation of αβT-lymphocytes occurs only in central (thymus) but not in peripheral lymphoid organs. However, evaluation of this parameter in an early stage of pregnancy (5th day) gave similar result: RAG-1 mRNA expression in thymocytes and its absence in lymph node T-cells. In the middle of pregnancy (11th day) RAG-1 mRNA expression was registered in thymus but also in para-aortal lymph nodes (in 70% of cases); on the 17th day expression was registered not only in lymph nodes involved in uterine drainage (60%) but in other lymph nodes as well (mainly in axillary lymph nodes, in 60% of cases). Analysis of pre-TCRα mRNA revealed similar tendencies (Fig. 1): lack of this transcript in peripheral T-lymphocytes in early stages of pregnancy and appearance of pre-TCRα mRNA expression (although less pronounced than RAG-1 mRNA) on the 11th day in para-aortal lymph nodes (in 40% of cases) and on the 17th day in both para-aortal lymph nodes (in 40% of cases) and in axillary lymph nodes (20% of cases) as well. These data indicate that from the middle of pregnancy the extrathymic differentiation of αβT-lymphocytes may also occur in mouse peripheral lymphoid organs. Appearance of peripheral unrearranged TCRα mRNA suggests that this process is related (at least partially) to thymocyte migration to lymph nodes because this marker is not expressed either in mature T-cell or bone marrow precursors [16].

During the second step of this study similar experiments have been carried out using CBA/J females mated with males of different strains: CBA/J × BALB/c (normal allogenic pregnancy), CBA/J × CBA/J (normal syngenic pregnancy), and CBA/J × DBA/2 (pregnancy with increased risk of abortion). In contrast to nonpregnant CBA/J females characterized by thymic RAG-1 mRNA expression only (Fig. 2), in pregnant mice RAG-1 mRNA transcript was also detected in peripheral T-lymphocytes on the 9th day. This was typical not only for normal pregnancy (CBA/J × BALB/c, CBA/J × CBA/J), but also for mating characterized by the increased risk of abortion (CBA/J × DBA/2). No principal differences have been found: in all variants of mating the most stable RAG-1 mRNA expression was detected in para-aortal lymph nodes (in 60-100% cases) and to a lesser extent in axillary (~40%) and mesenteric (~10%) lymph nodes. Similar results were obtained using pure population of αβT-lymphocytes isolated from corresponding lymph nodes (Fig. 2). This rules out the possible influence of B-lymphocytes or γδT-lymphocytes, which present in lymph nodes and normally express RAG proteins [4, 19].

Results of experiments with Swiss mice coincided with the results obtained using linear animals and mode

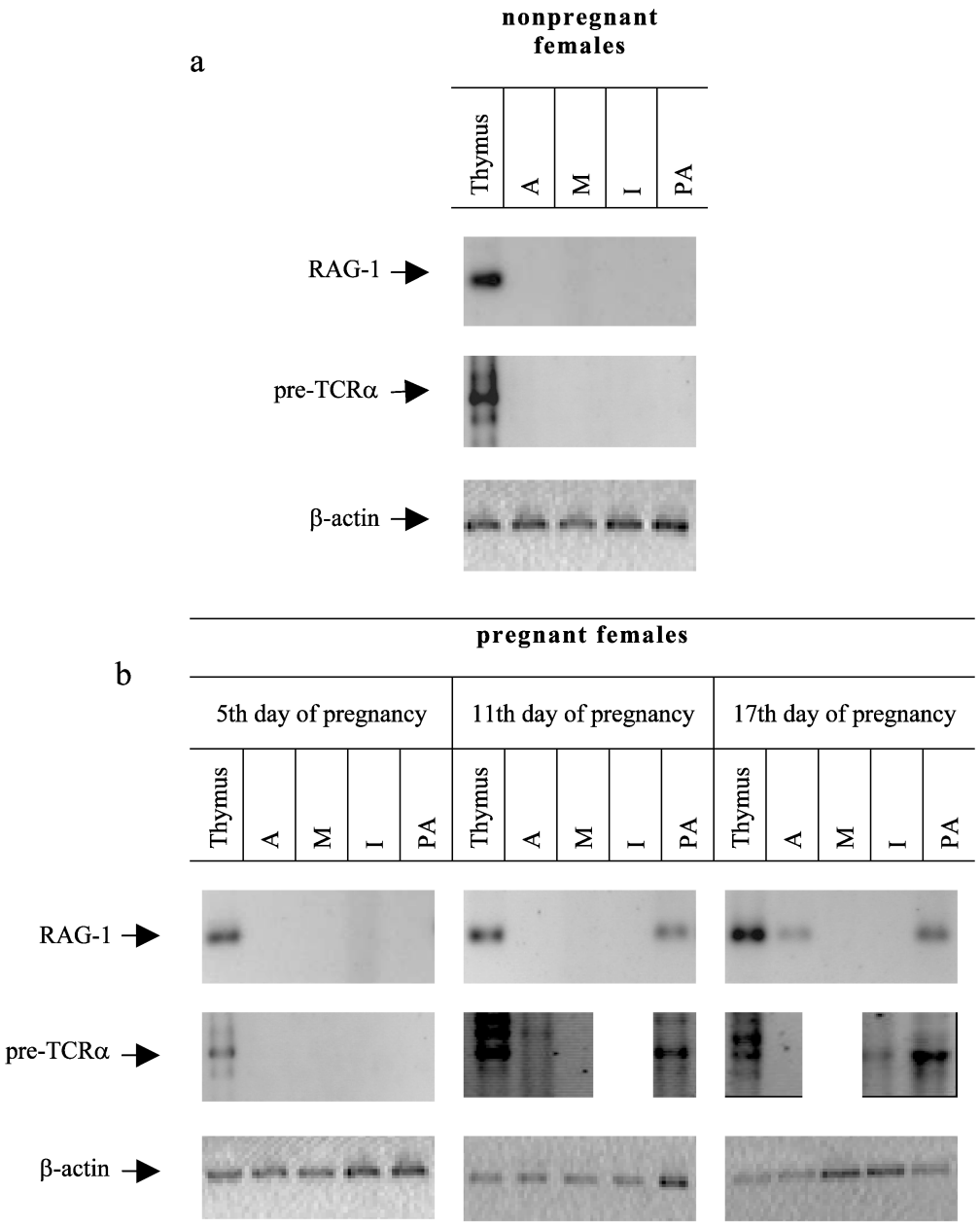

Fig. 1. Expression of RAG-1 mRNA and pre-TCRα mRNA in thymic and lymph node T-cells of nonpregnant (a) and pregnant (b) Swiss mice. Results of one of 8-12 experiments. Here and in Fig. 2 the following abbreviations have been used: PA) para-aortal; A) axillary; M) mesenteric; I) inguinal (lymph nodes).

of RAG-1 mRNA expression did not depend of mating variants. The latter suggests that this is a universal process (in spite of its limitation) and high frequency of abortions seen in the mating combination CBA/J × DBA/2 is not related to RAG-1 mRNA expression.

Existence of rearrangement of the antigen receptor in lymph node T-cells seen in pregnancy has at least two logical explanations. The first one implies migration of immature thymocytes to peripheral lymphoid organs (induced by various pregnancy-associated factors), where they finish their development (Fig. 3). Expression of pre-TCRα (normally detected only in early thymic precursors) in peripheral T-cell supports this mechanism [16]. The second mechanism suggests activation of recently detected process known as "antigen receptor revision"; this process is accompanied by repeated induction of rearrangement of antigen receptor chains in mature peripheral T lymphocytes resulting in formation of TCR of a new specificity [22]. It is also possible that both mechanisms contribute to this process. Such process may

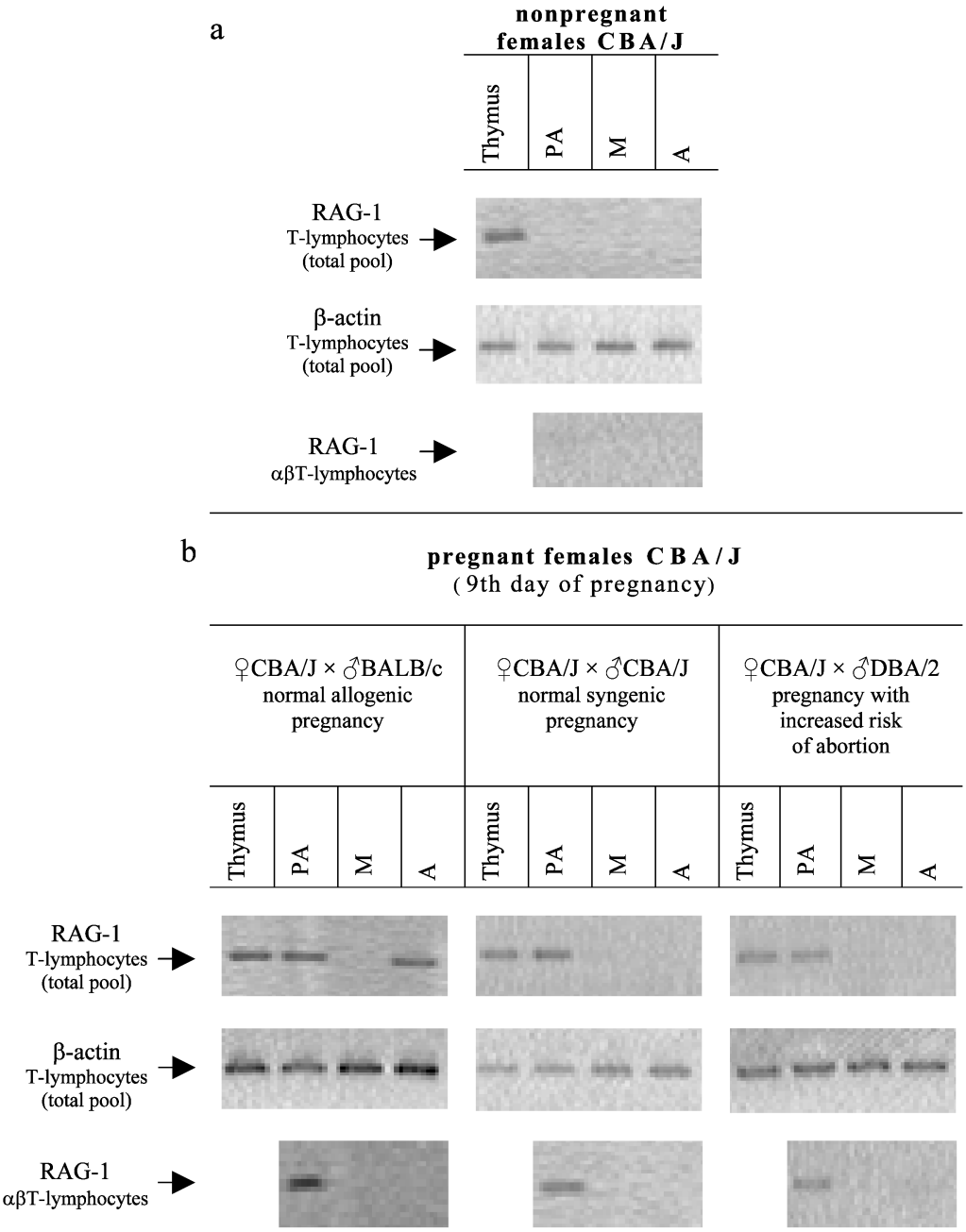

Fig. 2. Expression of RAG-1 mRNA in thymic and lymph node T-cells of nonpregnant (a) and pregnant (9th day) (b) CBA mice. Results of one of 5-12 experiments.

potentially be induced by members of the interleukin-6 family such as oncostatin M (OsM) [13] and/or leukemia inhibitory factor (LIF) [23] and estrogens [14], which can also activate processes of extrathymic differentiation of αβT-cells in mice during exogenous administration [13, 14] or in the case of transgenic expression [23]. Also, certain evidence exists that in OsM transgenic animals hemopoietic and stromal cells of lymph nodes may present tissue-specific antigens as effectively as thymic epithelial cells; this provides clonal selection of peripheral T-lymphocytes during their maturation [24]. Thus, in the case of OsM-dependent activation of extrathymic differentiation, lymph nodes exhibit the thymic functions.

In suggesting biological importance of this phenomenon, one should take into consideration that it takes place under conditions of pregnancy, accompanied by fetus expression of foreign (father's) antigens; this suggests involvement of complex mechanisms regulating the mother's immune system, which prevents rejection reactions. Elucidation of these mechanisms has not yet been

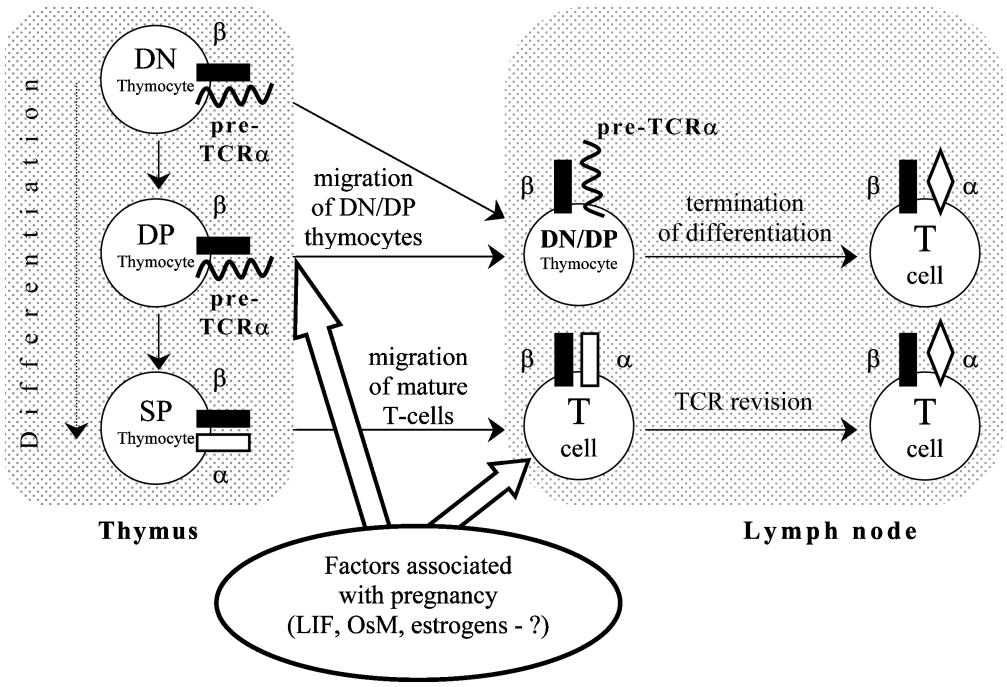

Fig. 3. Hypothetical mechanisms underlying appearance of RAG-1 mRNA and pre-TCRα in population of peripheral T-lymphocytes during pregnancy. DN) double negative thymocyte ($CD4^-CD8^-$); DP) double positive thymocyte ($CD4^+CD8^+$); LIF) leukemia inhibitory factor; OsM) oncostatin M; SP) single positive thymocyte ($CD4^+CD8^-$ or $CD4^-CD8^+$).

finished. Special interest is attracted to activation of rearrangement processes in lymph nodes involved in uterine drainage, which has been demonstrated in this study. It is possible that this activation consists in both compensation pregnancy-induced thymic atrophy and also local correction of antigen recognizing repertoire of T-lymphocytes due to appearance of new (fetal and placental) antigens in the body. Perhaps this represents a new, previously unknown mechanism of formation of tolerance to these antigens.

The second T-cell subpopulation (γδT-lymphocytes, differentiating outside thymus and forming its own antigen recognizing repertoire in the cell environment, which differs from the thymic environment [4, 25, 26]), is especially important during pregnancy. These cells mainly control local immune reactions, particularly at the fetal maternal interphase. At the same time, the immune response to fetal antigens also occurs at the systemic level (due to penetration of fetal and placental antigen into the mother's blood circulation [27, 28]). The central role in systemic immune reactions belongs to αβT-lymphocytes. However, cells undergoing clonal selection in the thymus and lacking contacts with fetal antigens at this stage cannot exhibit tolerance to these antigens on the periphery. The hypothesis on possible correction of the antigen-recognizing repertoire of αβT-lymphocytes during pregnancy may solve one of the main problems of reproductive immunology: why the maternal immune system is tolerant to foreign fetal antigens but at the same time is rather reactive and mobile with respect to other foreign antigens. However, the solution of this problem requires comparison of repertoire of TCR RAG-1^+/pre-$TCRa^+$-T-cells with corresponding repertoire of the T-lymphocytes differentiating in the thymus.

This work was supported by a grant from the Russian Foundation for Basic Research-Ural (No. 04-04-96064).

REFERENCES

1. Yarilin, A. A., Pinchuk, V. G., and Grinevich, Yu. A. (1991) *Structure of Thymus and T-Lymphocyte Differentiation* [in Russian], Naukova Dumka, Kiev.
2. Fehling, H. J., and von Boehmer, H. (1997) *Curr. Opin. Immunol.*, **9**, 263-275.
3. Robey, E. A., and Fowlkes, B. J. (1998) *Curr. Opin. Immunol.*, **10**, 181-187.
4. Mincheva-Nilsson, L., Kling, M., Hammarstrem, S., Nagaeva, O., Sundqvist, K. G., Hammarstrem, M. L., and Baranov, V. (1997) *J. Immunol.*, **159**, 3266-3277.
5. Shimizu, T., Kawamura, T., Miyaji, T., Oya, H., Bannai, M., Yamamoto, S., Weerasinghe, A., Halder, L. C., Watanabe, H., Hatakeyama, K., and Abo, T. (2000) *Scand. J. Immunol.*, **51**, 285-292.
6. Seki, S., Abo, T., Ohteki, N., Sugiura, K., and Kumagai, K. (1991) *J. Immunol.*, **147**, 1214-1221.
7. Abo, T. (2001) *Int. Immunol.*, **1**, 1261-1273.

8. Kadena, T., Matsuzaki, G., Fujise, S., Kishihara, K., Takimoto, H., Sasaki, M., Beppu, M., Nakamura, S., and Nomoto, K. (1997) *Immunology*, **91**, 511-519.
9. Lamontagne, L., Massicotte, E., and Page, C. (1997) *Immunology*, **90**, 402-411.
10. Ohteki, T., Okyuama, R., Seki, S., Abo, T., Sugiura, K., Kusumi, A., Ohmori, T., Watanabe, H., and Kumagai, K. (1992) *J. Immunol.*, **149**, 1562-1570.
11. Rijhsinghani, A. G., Bhatia, S. K., Tygrett, L. T., and Waldschmidt, T. J. (1996) *Am. J. Reprod. Immunol.*, **35**, 523-528.
12. Shimomiya, N., Tsuru, S., Tsugita, M., Katsura, U., Takemura, T., Rokutanda, M., and Homoto, K. (1991) *J. Clin. Lab. Immunol.*, **34**, 11-22.
13. Boileau, C., Houde, M., Dulude, G., Clegg, C. H., and Perreault, C. (2000) *J. Immunol.*, **164**, 5713-5720.
14. Okuyama, R., Abo, T., Seki, S., Ohteki, T., Sugiura, A., Kusumi, A., and Kumagai, K. (1992) *J. Exp. Med.*, **175**, 661-669.
15. Turka, L., Schatz, D., Oettinger, M., Chun, J., Gorka, C., Lee, K., McCormack, W., and Thompson, C. (1991) *Science*, **16**, 778-781.
16. Bruno, L., Rocha, B., Rolink, A., von Boehmer, H., and Rodewald, H. R. (1995) *Eur. J. Immunol.*, **25**, 1877-1882.
17. Chaouat, G., Kiger, N., and Wegmann, T. G. (1983) *J. Reprod. Immunol.*, **5**, 389-392.
18. Lin, Y., Zeng, Y., Zhao, J., Zeng, S., Huang, J., Feng Z., Di, J., and Zhan, M. (2004) *J. Reprod. Immunol.*, **64**, 133-143.
19. Hikida, M., Mori, M., Takai, T., Tomochika, K.-I., Hamatani, K., and Ohmori, H. (1996) *Science*, **274**, 2092-2094.
20. Vaitaitis, G. M., Poulin, M., Sanderson, R. J., Haskins, K., and Wagner D. H., Jr. (2003) *J. Immunol.*, **170**, 3455-3459.
21. Corcoran, L., Ferrero, I., Vremec, D., Lucas, K., Waithman, J., O'Keeffe, M., Wu, L., Wilson, A., and Shortman, K. (2003) *J. Immunol.*, **170**, 4926-4932.
22. Kuklina, E. M. (2006) *Biochemistry* (Moscow), **71**, 827-837.
23. Shen, M. M., Skoda, R. C., Cardiff, R. D., Campos-Torres, J., Leder, P., and Ornitz, D. M. (1994) *EMBO J.*, **13**, 1375-1385.
24. Blais, M.-E., Louis, I., and Perreault, C. (2006) *Immunol. Rev.*, **209**, 103-114.
25. Hayakawa, S., Shiraishi, H., Saitoh, S., and Satoh, K. (1996) *Am. J. Reprod. Immunol.*, **35**, 233-238.
26. Shirshev, S. V. (1999) *Mechanisms of Immune Control of Reproduction Processes* [in Russian], Ural Branch of the Russian Academy of Sciences, Ekaterinburg.
27. Heyborne, K., Fu, Y.-X., and Nelson, A. (1994) *J. Immunol.*, **153**, 2918-2926.
28. Van Wijk, I. J., Griffioen, S., Tjoa, M. L., Mulders, M. A., van Vugt, J. M., Loke, Y. W., and Oudejans, C. B. (2001) *Am. J. Obstet. Gynecol.*, **184**, 991-997.