A.M.Kamyshny
T.M.Prozorova
V.A.Kamyshna

Zaporizhzhia State Medical University

UDC 612.438.017.1:616.379-008.64-092.9

THE INFLUENCE OF EXPERIMENTAL GESTATIONAL DIABETES ON EXPRESSION OF AIRE mRNA AND CHARACTER OF DIFFERENTIATION OF FOXP3+ - CELLS IN MESENTERIC LYMPH NODES IN THE OFFSPRING

The study was conducted as the part of research work "Role of impaired relations of lymphoid and epithelial compartments of mucosal immune system in the development of experimental pathology" (state registration number 0112U005642).

Key words: experimental gestational diabetes, mesenteric lymph nodes, Aire, Treg.

ABSTRACT. Background. Formation of immunological tolerance to self-antigens is an important mechanism that prevents development of the autoimmune diseases. **Objective.** With the help of molecular genetic and immunofluorescence techniques to investigate the effects of experimental gestational diabetes on the level of mRNA expression of autoimmune regulator Aire and differentiation features of Foxp3+ cells in mesenteric lymph nodes in the offspring of Wistar rats. **Methods.** To determine the level of Aire mRNA RT-PCR was performed in real-time by thermocycler CFX96™ Real-Time PCR Detection Systems («Bio-RadLaboratories, Inc», USA). The relative level of gene expression were studied with rat reference genes GAPDH by the method ΔΔCt. Statistical analysis were conducted using available software «Bio-Rad CFX Manager 3.1» (Bio-Rad, USA). The immunopositive Foxp3+-lymphocytes were determined using an indirect immunofluorescence technique with using a monoclonal rat antibody. **Results.** The offspring of experimental gestational diabetes rats showed the reduction of autoimmune regulator Aire mRNA in 2,3-8,1 times ($p < 0,05$) in mesenteric lymph node cells compared to control animals. The observed decrease in the transcriptional activity of Aire is accompanied by reduction in the number of regulatory Foxp3+ lymphocytes in mesenteric lymph node in the offspring of rats with experimental gestational diabetes, as well as reduction of concentration of the transcription factor Foxp3 in lymphocytes of cortical plateau. **Conclusion.** The revealed changes evidence the abuse of formation of peripheral immunological tolerance and can trigger the development of autoimmune disease in the offspring of mothers with experimental gestational diabetes.

Received: 18.05.2015
Accepted: 06.06.2015

alexkamyshny@yandex.ru

Citation:
Kamyshny AM, Prozorova TM, Kamyshna VA. [The influence of experimental gestational diabetes on expres-sion of Aire mRNA and character of differentiation of Foxp3+ - cells in mesenteric lymph nodes in the offspring]. Morphologia. 2015;9(2):29-35. Russian.

References

1. Akirav EM, Ruddle NH, Herold KC. The role of AIRE in human autoimmune disease. Nat Rev Endocrinol. 2011;7(1):25-33. PMID: 21102544.
2. Laan M, Peterson P. The many faces of Aire in central tolerance. Front Immunol. 2013;4:326. PMID: 24130560.
3. Semana G, Gausling R, Jackson RA, Hafler DA. T cell autoreactivity to proinsulin epitopes in diabetic patients and healthy subjects. J Autoimmun. 1999;12:259-67. Cited in PubMed; PMID: 10330297.
4. Narendran P, Neale AM, Lee BH, Ngui K, Steptoe RJ, Morahan G, Madsen O, Dromey JA, Jensen KP, Harrison LC. Proinsulin is encoded by an RNA splice variant in human blood myeloid cells. Proc Natl Acad Sci USA. 2006;103(44):16430-5. PMID: 17053071.
5. Kojima H, Fujimiya M, Matsumura K, Nakahara T, Hara M, Chan L. Extrapancreatic insulin-producing cells in multiple organs in diabetes. Proc Natl Acad Sci USA. 2004;101(8):2458-63. PMID: 14983031.
6. Shevach E, Thornton A. tTregs, pTregs, and iTregs: similarities and differences. Immunol Rev. 2014;259(1):88-102. doi: 10.1111/imr.12160. PMID: 24712461.
7. Macpherson A, Smith K. Mesenteric lymph nodes at the center of immune anatomy. J Exp Med. 2006;203(3):497-500. PMID: 16533891.
8. Aschenbrenner K, D'Cruz LM, Vollmann EH, Hinterberger M, Emmerich J, Swee LK. Selection of Foxp3+ regulatory T cells specific for self antigen expressed and presented by Aire+ medullary thymic epithelial cells. Nat Immunol. 2007;8(4):351-8. PMID: 17322887.
9. Hinterberger M, Aichinger M, da Costa O, Voehringer D, Hoffmann R, Klein L. Autonomous role of medullary thymic epithelial cells in central CD4(+) T cell tolerance. Nat Immunol. 2010;11(6):512-9. doi: 10.1038/ni.1874. PMID: 20431619.
10. Nagamine K, Peterson P, Scott HS, Kudoh J, Minoshima S, Heino M, Krohn KJ, Lalioti MD, Mullis PE, Antonarakis SE, Kawasaki K, Asakawa S, Ito F, Shimizu N. Positional cloning of the APECED gene. Nat Genet. 1997;17(4):393-8. PMID: 9398839.
11. Kont V, Laan M, Kisand K, Merits A, Scott HS, Peterson P. Modulation of Aire regulates the expression of tissue-restricted antigens. Mol Immunol. 2008;45(1):25-33. PMID: 17599412.
12. Poliani PL, Kisand K, Marrella V, Ravanini M, Notarangelo LD, Villa A, Peterson P, Facchetti F. Human peripheral lymphoid tissues contain au-toimmune regulator-expressing dendritic cells. Am J Pathol. 2010;176(3):1104-12. doi: 10.2353/ajpath.2010.090956. PMID: 20093495.
13. Suzuki E, Kobayashi Y, Kawano O, Endo K, Haneda H, Yukiue H, Sasaki H, Yano M, Maeda M, Fujii Y. Expression of AIRE in thymocytes and peripheral lymphocytes. Autoimmunity. 2008;41(2):133-9. doi: 10.1080/08916930701773941. PMID: 18324482.
14. Gardner JM, Devoss JJ, Friedman RS, Wong DJ, Tan YX, Zhou X, Johannes KP, Su MA, Chang HY, Krummel MF, Anderson MS. Deletional tolerance mediated by extrathymic Aire-expressing cells. Science. 2008;321(5890):843-7. doi: 10.1126/science.1159407. PMID: 18687966.

15. Fletcher AL, Lukacs-Kornek V, Reynoso ED, Pinner SE, Bellemare-Pelletier A, Curry MS, Collier AR, Boyd RL, Turley SJ. Lymph node fibroblastic reticular cells directly present peripheral tissue antigen under steady-state and inflammatory conditions. J Exp Med. 2010;207(4):689-97. doi: 10.1084/jem.20092642. PMID: 20308362

16. Hubert FX, Kinkel SA, Webster KE, Cannon P, Crewther PE, Proeitto AI, Wu L, Heath WR, Scott HS. A specific anti-Aire antibody reveals Aire expression is restricted to medullary thymic epithelial cells and not expressed in periphery. J Immunol. 2008;180(6):3824-32. PMID: 18322189.

17. Zheng X, Yin L, Liu Y, Zheng P. Expression of tissue-specific autoantigens in the hematopoietic cells leads to activation-induced cell death of autoreactive T cells in the secondary lymphoid organs. Eur J Immunol. 2004;34(11):3126-34. PMID: 15368272.

18. Cohen JN, Guidi CJ, Tewalt EF, Qiao H, Rouhani SJ, Ruddell A, Farr AG, Tung KS, Engelhard VH. Lymph node-resident lymphatic endothelial cells mediate peripheral tolerance via Aire-independent direct antigen presentation. J Exp Med. 2010;207(4):681-8. doi: 10.1084/ jem.20092465. PMID: 20308365.

19. Yang S, Fujikado N, Kolodin D, Benoist C, Mathis D. Immune tolerance. Regulatory T cells generated early in life play a distinct role in maintaining self-tolerance. Science. 2015;348(6234):589-94. doi: 10.1126/science.aaa7017. PMID: 25791085.

20. Daniely D, Kern J, Cebula A, Ignatowicz L. Diversity of TCRs on natural Foxp3+ T cells in mice lacking Aire expression. J Immunol. 2010;184(12):6865-73. PMID: 20483761.

21. Malchow S, Leventhal DS, Nishi S, Fischer BI, Shen L, Paner GP, Amit AS, Kang C, Geddes JE, Allison JP, Socci ND, Savage PA. Aire-dependent thymic development of tumor-associated regulatory T cells. Science. 2013;339(6124):1219-24. doi: 10.1126/science.1233913. PMID: 23471412.

22. Sun J, Fu H, Wu J, Zhu W, Li Y, Yang W. Macrophages overexpressing Aire induce CD4+Foxp3+ T cells. Mol Med Rep. 2013;7(1):159-65. doi: 10.3892/mmr.2012.1150. PMID: 23128958.

23. Gardner JM, Metzger TC, McMahon EJ, Au-Yeung BB, Krawisz AK, Lu W, Price JD, Johannes KP, Satpathy AT, Murphy KM, Tarbell KV, Weiss A, Anderson MS. Extrathymic Aire-expressing cells are a distinct bone marrow-derived population that induce functional inactivation of $CD4^+$ T cells. Immunity. 2013; 39(3):560-72. doi: 10.1016/j.immuni.2013.08.005. PMID: 23993652.

24. Pomié C, Vicente R, Vuddamalay Y, Lundgren BA, van der Hoek M, Enault G, Kagan J, Fazilleau N, Scott HS, Romagnoli P, van Meerwijk JP. Autoimmune regulator (AIRE)-deficient CD8+CD28low regulatory T lymphocytes fail to control experimental colitis. Proc Natl Acad Sci USA. 2011;108(30):12437-42. doi: 10.1073/pnas.1107136108. PMID: 21746930.

25. Nakajima A, Negishi N, Tsurui H, Kado-waki-Ohtsuji N, Maeda K, Nanno M, Yamaguchi Y, Shimizu N, Yagita H, Okumura K, Habu S. Commensal bacteria regulate thymic Aire expression. PLoS One. 2014;9(8):e105904. doi: 10.1371/journal.pone.0105904. PMID: 25157574.

26. Zhu W, Yang W, He Z, Liao X, Wu J, Sun J, Yang Y, Li Y. Overexpressing autoimmune regulator regulates the expression of toll-like receptors by interacting with their promoters in RAW264.7 cells. Cell Immunol. 2011;270(2):156-63. doi: 10.1016/j.cellimm.2011.05.002. PMID: 21628060.