

- Gillespie W., Paulson J.C., Kelm S., Pang M., Baum L.G. 1993. Regulation of alpha 2,3-sialyltransferase expression correlates with conversion of peanut agglutinin (PNA)+ to PNA-phenotype in developing thymocytes. *J. Biol. Chem.* V. 268. P. 3801.
- Hellström U., Hammarström S., Dillner M.L., Perlmann H., Perlmann P. 1976. Fractionation of human blood lymphocytes on Helix pomatia A haemagglutinin coupled to sepharose beads. *Scand. J. Immunol.* V. 5. P. 45.
- Iwamori M., Iwamori Y. 2005. Changes in the glycolipid composition and characteristic activation of GM3 synthase in the thymus of mouse after administration of dexamethasone. *Glycoconj. J.* V. 22. P. 119.
- Kishimoto H., Surh C.D., Sprent J. 1995. Upregulation of surface markers on dying thymocytes. *J. Exp. Med.* V. 181. P. 649.
- Krishna M., Varki A. 1997. 9-O-Acetylation of Sialomucins: A Novel Marker of Murine CD4 T Cells that Is Regulated during Maturation and Activation. *J. Exp. Med.* V. 185. P. 1997.
- Lee C.K., Kim J.K., Kim Y., Lee M.K., Kim K., Kang J.K., Han S.S. 2001. Generation of macrophages from early T progenitors *in vitro*. *J. Immunol.* V. 166. P. 5964.
- Lesley J., Schulte R., Trotter J., Hyman R. 1988. Qualitative and quantitative heterogeneity in Pgp-1 expression among murine thymocytes. *Cell. Immunol.* V. 112. P. 40.
- London J., Berrih S., Bach J.F. 1978. Peanut agglutinin I. A new tool for studying T lymphocyte subpopulations. *J. Immunol.* V. 121. P. 438.
- Morris R.G., Hargreaves A.D., Duvall E., Wylie A.H. 1984. Hormone-induced cell death. 2. Surface changes in thymocytes undergoing apoptosis. *Am. J. Pathol.* V. 115. P. 426.
- Palmer E. 2003. Negative selection – clearing out the bad apples from the T-cell repertoire. *Nat. Rev. Immunol.* V. 3. P. 383.
- Pilate Y., Bignon J., Lambré C.R. 1993. Sialic acids as important molecules in the regulation of the immune system: pathophysiological implications of sialidases in immunity. *Glycobiol.* V. 3. P. 201.
- Raedler A., Raedler E., Becker W.M., Arndt R., Thiele H.G. 1982. Subcapsular thymic lymphoblasts expose receptors for soy bean lectin. *Immunology.* V. 46. P. 321.
- Reitsma S., Slaaf D.W., Vink H., van Zandvoort M.A., oude Egbrink M.G. 2007. The endothelial glycocalyx: composition, functions, and visualization. *Eur. J. Physiol.* V. 454. P. 345.
- Roth J., Taatjes D.J., Lucocq J.M., Weinstein J., Paulson J.C. 1985. Demonstration of an Extensive Trans-tubular Network Continuous with the Golgi Apparatus Stack That May Function in Glycosylation. *Cell.* V. 43. P. 287.
- Sawicka M., Stritesky G., Reynolds J., Abourashchi N., Lythe G., Molina-Paris C., Hogquist K. 2014. From pre-DP, post-DP, SP4, and SP8 thymocyte cell counts to a dynamical model of cortical and medullary selection. *Front. Immunol.* V. 15. P. 1.
- Schulte B.A., Spicer S.S. 1985. Histochemical methods for characterizing secretory and cell surface sialoglycoconjugates. *J. Histochem. Cytochem.* V. 33. P. 427.
- Scollay R.G., Butcher E.C., Weissman I.L. 1980. Thymus cell migration: quantitative aspects of cellular traffic from the thymus to the periphery in mice. *Eur. J. Immunol.* V. 10. P. 210.
- Screpanti I., Morrone S., Meco D., Santoni A., Gulino A., Paolini R., Frati, L. 1989. Steroid sensitivity of thymocyte subpopulations during intrathymic differentiation. Effects of 17 beta-estradiol and dexamethasone on subsets expressing T cell antigen receptor or IL-2 receptor. *J. Immunol.* V. 142. P. 3378.
- Sinclair C., Bains I., Yates A.J., Seddon B. 2013. Asymmetric thymocyte death underlies the CD4:CD8-T-cell ratio in the adaptive immune system. *PNAS.* V. 110. P. 2905.
- Stobo J.D. 1972. Phytohemagglutinin and concanavalin A: Probes for murine “T” cell activation and differentiation. *Immunol. Rev.* V. 11. P. 60.
- Surh C.D., Sprent J. 1994. T-cell apoptosis detected *in situ* during positive and negative selection in the thymus. *Nature.* V. 372. P. 100.
- Wu W., Punt J.A., Granger L., Sharrow S.O., Kearse K.P. 1997. Developmentally regulated expression of peanut agglutinin (PNA)-specific glycans on murine thymocytes. *Glycobiol.* V. 7. P. 349.
- Wylie A.H. 1980. Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. *Nature.* V. 284. P. 555.

## The Experience in Lectins Application to Assess Changes in the Carbohydrate Composition of Murine Thymocytes Glycocalyx in the Early and Late Apoptotic Stages

M. K. Serebriakova<sup>a</sup>, I. V. Kudryavtsev<sup>a</sup>, E. Balkan<sup>b</sup>, and A. V. Polevshchikov<sup>a,\*</sup>

<sup>a</sup>Institute of Experimental Medicine, Saint Petersburg, 197376 Russia

<sup>b</sup>Molecular Biology Section, Manisa Celal Bayar University, Manisa, 45030 Turkey

\*e-mail: ALEXPOL512@yandex.ru

The apoptosis process is an important element in the maturation and differentiation of T-lymphocytes. The work is devoted to the analysis of changes in the composition of CBA mice thymocytes glycocalyx oligosaccharides during the hydrocortisone induced apoptosis. A panel of 23 fluoresceinisothiocyanate-labeled lectins specific for mannose, mannose and glucose, galactose, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, fucose and N-acetylneuraminic acid residues was used. Flow cytometry was the method for evaluation the binding of lectins to thymocytes of intact mice, as well as mice after administration of hydrocortisone. Based on the results of TMRM and 7-AAD staining, the cells were divided into living thymocytes, cells in early and late apoptosis. It has been established that living cells carry carbohydrates on the surface of glycocalyx containing terminal residues of galactose and N-acetyl-D-galactosamine. With the transition of thymocytes to late apoptosis, the binding of all lectins increases, except for fucose-specific. The external glycocalyx structures of living thymocytes are low in density and contain groups of oligosaccharides with N-acetyl-D-galactosamine and D-galactose in the terminal position. The near-membrane layer of glycocalyx is characterized by a high density and a wide variety of oligosaccharide structures that persist at the stage of late apoptosis of thymocytes. The results indicate nonuniform density and heterogeneity of oligosaccharides in glycocalyx, a significant part of which is lost in early apoptosis.

**Keywords:** thymocytes, apoptosis, lectins, glycocalyx carbohydrates