THE ROLE OF NEUROGLIA IN THE HUMAN VITREOUS

Yana Sadovaya[1], Galina Nikolayenko[1], Galina Reva[1], Tatiyana Lemeshko[1], Nataliya Kostyuk[1], Kseniya Albrandt[1], Tatiyana Poleshchuk[1], Anna Zudina[1], Ivan Reva[1,2]

[1] Far Eastern Federal University, Vladivostok, Russia
[2] International Medical Education and Research Center (IMERC) Niigata, Japan
e-mail: avers2@yandex.ru.; RevaGal@yandex.ru

The research was supported by the FEFU Science Foundation under the state task 17.5740.2017/6.7.

Yana Sadovaya

Galina Nikolayenko

Anna Zudina

Nataliya Kostyuk

RELEVANCE

The human eye of the eye is the least studied structure. In this case, any change in the vitreous is a universal damaging factor, both for the induction of the pathology of the lens and the retina, so the relevance of the study of the histophysiology of the vitreous humor is beyond doubt [4, 15]. The importance of increasing intraocular pressure in glaucoma remains a subject of acute debate, as is the induction of this pathology by abnormalities in the structure of the vitreous body (VB) [1, 7, 16]. At the same time, at the present stage the vitreous body of birds and animals [10, 18]. Numerous studies have raised a number of questions on the production of vitreous fibers, which require further in-depth analytical research [9, 14].

PURPOSE OF THE STUDY

To carry out phenotyping of the vitreous humor cells of the human eye.

MATERIAL AND METHODS

The study examined the material obtained from medical abortions, forensic medical autopsies of people who died from trauma; as well as material obtained during surgical interventions for post-traumatic enucleation of the eyes, at the age of 4 weeks of intrauterine life to 85 years. The studies were performed with the help of classical staining with hematoxylin and eosin, impregnation with silver by the Kahal, and also with the method of immune histochemistry with the use of markers CD68, CD163, CD204. The microscope Olympus Bx51 with a digital camera CD 25 and proprietary software was used to analyze the obtained material. Identification of immunocompetent cells was carried out according to the same scheme, in spite of different antigen localization in cellular structures: membranes, lysosomes, Golgi complex.

RESULTS OF THE STUDY

We have established that the vitreous body of the human eye (VB) consistently goes through the following stages of development: 1) embryonic mesenchymal cell 2) fetal vascular, which undergoes gradual apoptosis and desolation by the 8th month of the fetal period; 3) definitive, or final-fibrous connective tissue. By the time of birth VB is practically formed, finds of embryonic vessels can be associated with developmental abnormalities [8]. The complexity of the structural organization of the febrile vitreous body is not the same in its various departments. We have established that there are areas that are limited to membranes having a thickness of up to 20 μm and optically empty. It is also noted that the major larger fibers of the febrile core have a longitudinal direction. Small fibers with a thickness of 1 mm or less can be arranged obliquely, interwoven into larger ones. The fibrils of the core and the dissolved collagen along with hyaluronic acid

contribute to the preservation of the gel-like state and play the role of the soft skeleton VB. According to the ordered arrangement of fibers Vitreous can be attributed to the formed fibrous connective tissue. We noted that the fibers of the febrile skeleton are woven into the optic nerve shells in the zone of the disc, which ensures a high contact strength.

In analyzing the age-related changes in the vitreous fibrous core, it was found that the number of fibers gradually decreases, and this dynamics is initially inherent in fibers predominantly of the transverse direction. The network loops become irregular and uneven, which, in our opinion, may be due to the adaptation of vitreous cells to changes in physiological conditions and an increase in their synthetic activity. In the future, this process will be accompanied by depletion of the plastic properties of vitreous cells and, correspondingly, a decrease in the formation of fibers of the core of the vitreous. We have established that in the vitreous body, in addition to the febrile core, the cells are identified. Analysis of own results showed that the distances between vitreous cells can be different, this depends on their topography in VB. We observed an unequal number of cells in different sections of the VB.

Most of them in the zone bordering the ciliary body, as well as on the back surface of the vitreous. We found that the cells are located near the processes of the ciliary body, and near the lens, at the posterior pole of VB near the retina, or at a distance of 100 μm from the vitreoretinal border. Our results indicate that the number of cells per 1 mm^2 of the cut reaches an average of 7, and the cell density decreases toward the posterior pole and the center of the vitreous. We have noted the age-related dynamics of the decrease in the number of hyalocytes in humans. With age, the quantitative ratio of cells of different forms varies: at a young age, round ones predominate, with one or more nuclei located on the periphery of the VB; in adulthood, the number of stellate and spindle-shaped, with contacting processes, located also in the cortical layer increases; In the vitreous body of the eyes of older people, globular cells predominate, with a bubble in the cytoplasm, which are located centrally.

It is established that hyalocytes vary not only in size and shape, but also in relationships with the fibrous core.

We identified two types of cells of the vitreous body of the human eye: type I — cells of the vitreous producer, two species — A and B; II type of cells — leukocytes. According to our data, cells related to the collagenous core of the VB (group A) and freely located in the vitreous (group B) belong to the first type. Group A has a spindle-like shape, the cells seem to be soldered into the fibrous core of the vitreous. They resemble the Müller cells of the retina in their structure. Another group of cells — B, located in the vitreous freely, is characterized by large dimensions, light cytoplasm, basophilic nucleus and free location in the matrix VB. These cells have scalloped edges and look like fibroblasts and fibrocytes of loose fibrous connective tissue. The cell form is either round or oval, the cytoplasm passes into the intercellular substance without clear boundaries, the cell membrane is not identified. The kernels are round, ovoid, oblong, or bean-shaped, up to 8 μm in size. We assume that group A of the cell type is hyalocytes, possibly of a neuroglia nature. Group B of type I cells, in our opinion, refers to connective tissue originating from the mesenchyme surrounding the vitreous embryonic vessels - fibrocystic and fibroblasts. Type I cells are the producers of the intercellular matrix vitreous, its febrile core and the basic substance. According to our data, the II type of cells morphologically corresponds to the leukocyte pool. Since they are naturally leukocytes, they easily change shape, forming processes. In the center of the vitreous humor, where there is more moisture, these cells undergo a vacillation, turning into a bubble. Their presence in the predominant quantity in people of older age groups is due to the fact that the vitreous body of the elderly is more diluted than in children. You can treat bladder cells as a degenerative form of leukocytes [5]. We have established that the II type of cells located loosely between the loops of the collagen-fibrous backbone, derivatives of the stem cell of the blood (SCB). With the help of immune histochemistry, we found out that in the vitreous body of the human eye there are different cell differentiations of blood stem cells. Some of the cells in our study are labeled CD163, which indicates their monocytes origin and phagocytic function. Cells with the labeling of fibroblasts, mast cells CD204, as well as CD68 cells, which mark the antigen-presenting function and belonging to interstitial dendritic cells, are identified. This indicates the possibility of reparative regeneration after vitrectomy after autologous and heterotransplantation of hyalocytes.

We have established that the proliferative activity of hyalocytes changes during the development of the eye and is dependent on the stage of ontogenesis. In the embryonic and fetal period, the proliferative activity of the vitreous humor cells is high enough, after birth it decreases, then is at approximately the same level until age 45. After 45 years, the figures of mitosis in vitreous cells were observed as a very rare phenomenon

The age-related involution of the vitreous humor consists in the formation in it of various sizes of cavities containing liquid fractions. Involution changes

include filamentous destruction, which manifests itself after 20 years, growing after 40 years. It is connected, in our opinion, with the age-related decrease in the synthetic and proliferative properties of hyalocytes — the own cells of the vitreous body of the human eye.

DISCUSSION OF THE RECEIVED DATA

Many authors argue that the vitreous does not have a structure that can be studied microscopically. Worst considered vitreous as a secret of cells and considered the detected fibers as an artifact [1]. The theory of the structure of VB alveolar, lamellar, radial-sectoral, according to our data, are untenable, are insolvent, in contrast to febrile, with which the results obtained by us agree. In this case, the treatment of fibrous vitreous body cannot be unambiguous. The location, thickness and direction of vitreous fibers suggest that they are not only a supporting structure, but also participation in the hydrodynamics of the eye, and also in visual functions through the correct distribution of the light flux in the sagittal direction [6, 12].

The widespread hypotheses of Meyer, Smith and Gallardo on the production of hyaluronic acid in vitreous by cells of the ciliary body (VB) were based on data on the absence of CT cells. Studies to determine the concentration of hyaluronic acid in the anterior, posterior and peripheral parts of the CT showed that its content near the ciliary body is only half the concentration present in the posterior part of the vitreous. Contrary to this data, Snead D. R. J., James S., Snead M. P. (2008), who did not find a definite regularity in the quantitative ratio of hyaluronic acid in the center and on the periphery VB [17].

The opinion of the researchers who state that there is no febrile core in the vitreous is based on the results obtained with the aid of the sampling of material with needles with a very small diameter. This could cause a misconception about the structure of the VB, since, of course, that in this case the elements of the core could not get into the composition of the extract, but only the dissolved collagen.

The contemporary state of the question of the structure of the vitreous body of the human eye indicates the absence of convincing morphological data in favor of the presence of hyalocytes in it, therefore it is the subject of heated discussions. Wayne J. (2003) did not find cells in the vitreous body of the human eye [18]. Balazs E.A., Toth L.Z., Ozanics V. (1980) indicate that vitreous cells are present only in the cortical layer of the CT eye of animals [1].

The mesodermal concept of the origin of vitreous Sholler and Lieberculhn gave way to the ectodermal theory of Tornatola, linking its formation to the retina. Then, the concept of an analogy of the soft membrane preformed into the vitreous in the specific conditions of the eyes of Redslop and Gartner was confirmed [1]. Hypotheses, the authors of which tried to relate the production of the vitreous with cell elements, did not find confirmation. The transudation theory of Keccler's theory of the basal membrane of Frans, the secretory concept of Vensen and Granacher, the ectomesodermal Studnitska treat the vitreous as a product of transudation, secretion or preformation of embryonic vitreous vessels and intercellular substance. Balazs Hamburg, Seaman, Storm, Gartner identified hyalocytes and fibroblasts (fibroblasts) among the vitreous cells of the eye, Balazs considers them to be different cells, and Francois classifies them as one type of cell at different stages of development [1]. It should be noted that even today the genesis of hyalocytes has not been fully clarified. Boltz-Nitulescu G., Grabner G., Förster O. suggested that these CT cells are hematogenous derivatives [3, 9]. Balazs, Teng, Horven (1980) their occurrence is associated with embryonic vessels, neuroectoderma or with wandering cells such as leukocytes. Kita T., Hata A. (2007) refer them to microglia, counting the derivatives of monocytes [9].

Mashemer (1975) suggested the retinal pigment cells as a possible source of hyalocytes. The concept of Balazs, which confirms the bone marrow origin of the CT reticular cells, which later turns into hyalocytes, was previously controversial, thanks to markers of progenitor cells and activated monocytes, which are identified in vitreous and other eye structures [2, 11, 16].

In our opinion, the cells of the immunophagocytes link identified in our work play an important role in the physiological and reparative regeneration of the vitreous humor. We adhere to the conclusions of Newsome (1976) Francois (1978) on the neuroglial and neuromesenchymal origin of type I hyalocytes associated with the febrile skeleton and free, especially since, according to the literature, the discovery in them of the synthesis of reticules and collagen testifies in favor of their core production functions vitreous [7, 8]. This serves as an additional confirmation of the presence in the vitreous body of various cellular diferrons [13]. We can assume that the functioning of the organ of vision in conditions of immunodeficiency, inherent in its avascular structures, contributes to the performance of antigen presentation by residual cells of the vitreous.

Thus, according to our own data and literature data, the localization, morphological structure and functions of hyalocytes indicate their important role in the development and functioning of the VB and the organ of vision in general.

CONCLUSIONS

In our study, the vitreous humor of the human eye is formed by a special kind of formed connective tissue, represented by cellular differons of different origin and functions, consisting of a basic gel-like substance into which the fibrils of the correctly organized collagenous fiber frame are immersed.

REFERENCES

1. **Balazs E.A., Toth L.Z., Ozanics V.** Cytological studies on the developing vitreous as related to the hyaloid vessel system. Albrecht Von Graefes Arch. Klin. Exp. Ophthalmol. 1980;213(2):71–85.
2. **Bishop P.N.** Structural macromolecules and supramolecular organization of the vitreous gel. Prog. Retin eye res. (2000); 19, 323–344.
3. **Boltz-Nitulescu G., Grabner G., Förster O.** Macrophage-like properties of human hyalocytes. Adv. Exp. Med. Biol. 1979; 121B:223–8.
4. **Bryan P.** Toole Hyaluronan: from extracellular glue to pericellular cue Nature Reviews Cancer 4, 528–539 (30 June 2004) doi:10.1038/nrc1391.
5. **Els S., Gopferich A., Framme C., Heilman J.** Hyalocytes in tissue engineering. Adv. exp. Med. Biol. 2006.-Fisher J.P.(ed), 585.
6. **Hata Y, Sassa Y, Kita T, Miura M, Kano K, Kawahara S, Arita R, Nakao S, Shih JL, Ishibashi T.** Vascular endothelial growth factor expression by hyalocytes and its regulation by glucocorticoid.// Br. J. Ophthalmol. 2008 Nov;92(11):1540–4.
7. **François J, Verbraeken H, Victoria-Troncoso V.** Transplantation of hyalocytes after pars plans vitrectomy. Mod. Probl. Ophthalmol. 1979; 20:164–8.
8. **François J, Verbraeken H, Victoria-Troncoso V.** Hyalocytes transplantation after pars plana vitrectomy (author's transl) J. Fr. Ophtalmol. 1979, Aug-Sep;2(8-9):459–61.
9. **Kita T, Hata Y, Miura M, Kawahara S, Nakao S, Ishibashi T.** Functional characteristics of connective tissue growth factor on vitreoretinal cells. Diabetes. 2007, May;56(5):1421–8.
10. **Lei K, Wang N, Wang L, Wang B.** Morphological changes of the anterior segment after laser peripheral iridotomy in primary angle closure. Eye 23, 345-350 (7 December 2007) doi:10.1038.
11. **Li Songlin, Kelly Stephen J, Lamani Ejvis, Ferraroni Marta, Jedrzejas Mark J.** Structural basis of hyaluronan degradation by hyaluronate lyase. The EMBO Journal 19, 1228-1240 (15 March 2000).
12. **Los L.I.** The rabbit as an animal model for post-natal vitreous matrix differentiation and degeneration. Eye 22, 1223–1232 (7 March 2008).
13. **Qiao H., Hisatomi T., Sonoda K.N., Kura S., Sassa Y., Kinoshita S., Nacamura T., Sakamoto T., Ihibata T.** The characterisation of hyalocytes the origin, phenotype and turnover. Br. J. Ophthalmol. (2005), 89:513–517.
14. **Rasier R., Artunay O., Yuzbasioglu E., Sengul A., Bahcecioglu H.** The effect of intravitreal bevacizumab (avastin) administration on systemic hypertension. Eye (12 December 2008).
15. **Rittig M, Flügel C, Prehm P, Lütjen-Drecoll E.** Hyaluronan synthase immunoreactivity in the anterior segment of the primate eye. Graefes Arch Clin Exp Ophthalmol. 1993 Jun;231(6):313–7.
16. **Uehara M., Yamagava T., Kitagawa H.** Morphological studies of the hyalocytes in the chiken eye: scanning electron microscopy and inflammatory response after the intravitreous injection of carbon particles. J. Anatomy (1996); 188, (pt 3): 661–669.
17. **Snead D. R. J., James S., Snead M. P.** Pathological changes in the vitreoretinal junction 1: epiretinal membrane formation. Eye 22, 1310–1317 (14 March 2008).
18. **Wayne J. Streilein** Ocular immune privilege: therapeutic opportunities from an experiment of nature. Nature Reviews Immunology, 3, 879–889 (31 October 2003).