особенно эффективными оказались растворы бальзама в концентрации 10 и 15 мкл – их введение снизило величину выхода гемоглобина на 3,4 % и 3 % (рисунок 2).

Таким образом, отчетливо прослеживается протекторный эффект исследуемого препарата in vitro на состояние мембран эритроцитов студентов; экспериментальные данные свидетельствуют, что бальзам «Возрождение» может оказывать положительное воздействие на стабилизацию мембран. Механизм защитного действия бальзама «Возрождение» в отношении мембран, по-видимому, связан с его стабилизирующим влиянием на клеточные мембраны посредством синергизма действия аскорбиновой кислоты и органического йода, входящих в состав бальзама [6;7;8]. Уровень антиоксидантных свойств бальзама «Возрождение» позволяет рекомендовать его для профилактического применения при стрессорных ситуациях, вызывающих окислительное разрушение мембран при действии неблагоприятных факторов, в частности, при возможном развитии гипокинезии.

Таким образом, применение биологически активных препаратов (бальзама «Возрождение») – повышает резистентность мембраны эритроцитов. Анализ полученных результатов позволяет заключить, что уникальный состав исследуемых препаратов обеспечивает широкий спектр протекторных свойств и способствует повышению резистентности организма к действию стрессовых факторов, которые неизбежны в жизни студенческой молодежи.

Литература

1. Young, S., Woodside, J.V. Antioxidants in health and disease //J. Clin. Pathol. 2001. Vol. 54, № 3. P. 176-186.

2. Тутельян, В.А. Биологически активные добавки к пище: прошлое, настоящее и будущее // Тезисы второго международного симпозиума «Питание и здоровье. Биологически активные добавки к пище». М., 1996. С. 164-166.

3. Janisch, K.M., Milde, J., Schempp, H., Elstner, E.F. Vitamin c, vitamin e and flavonoids // Dev Ophthalmol. 2005. № 38. P. 59-69.

4. Покровский, А.А., Абрарова, А.А. К вопросу о перекисной резистентности эритроцитов // Вопр. питания. 1964. №16. С.44-49.

5. Мирошина, Т.Н., Мурзахметова, М.К., Утегалиева, Р.С. Корригирующее влияние индоламинов на состояние мембран эритроцитов при действии ионов кадмия // Вестник КазНУ. Сер. биол. 2002. № 3. С.80-86.

6. Branis, M, Burda, H. Effect of ascorbic acid on the numerical hair cell loss in noise exposed guinea pigs // Hear Res. 1988. V. 33. P. 137–140.

7. Derekoy, FS, Koken, T, Yilmaz, D, Kahraman, A, Altuntas, A. Effects of ascorbic acid on oxidative system and transient evoked otoacoustic emissions in rabbits exposed to noise //Laryngoscope. 2004. V. 114. P. 1775–1779.

8. McFadden, SL, Woo, JM, Michalak, N, Ding, D. Dietary vitamin C supplementation reduces noise-induced hearing loss in guinea pigs // Hear Res. 2005. V. 202. P. 200–208.

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Phenotypic analysis of human peripheral blood cd4+cd25+ treg cells binding and non-binding high molecular hyaluronan

Regulatory T cells (Treg), either natural or induced, suppress a variety of physiological and pathological immune responses and have great therapeutic potential. It has been established that population of Treg cells is very heterogenic, mechanism of their effect and participation in tumor progression, autoimmune or chronic inflammatory diseases is not completely clear. One of the key issues for understanding the defects in Treg cell functions under pathological processes is investigating the molecular basis of their functional state. Here we show a new approach to Treg cells investigation, which can discriminate a potentially capable to adhesion subset of Treg cells. Using this approach we characterized the population of freshly isolated hyaluronan-binding (HA+) CD4+CD25+ cells of peripheral blood of healthy donors. We showed that there was lower number of FoxP3+ and CD39+ cells in HA+ CD4+CD25+ subset than in HA- CD4+CD25+ one. The results suggest that in norm circulating natural Treg cells contain the subset capable to bind high molecular hyaluronan and thereby to be ready to control possible immune autoreactivity.

Keywords: T regulatory cells, hyaluronan, flow cytometry.

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Адамның шеткі аймақтық қанындағы жоғары молекулалы гиалурананмен байланысушы және байланыспаушы cd4+cd25+ treg жасушалардың фенотиптік талдауы

Т реттегіш жасушалар (Treg) әр түрлі физологиялық және патологиялық иммундық жауапқа қатысады және жоғары терапиялық потенциялы бар. Treg жасушалары өте гетерогенді екені анықталған, бірақ оның ісіктің өсуіндегі, аутоиммундық немесе хроникалық қабыну ауруындағы әсер механизімі толықтай анық емес. Патологиялық процесстегі Treg жасушасының функцясындағы кемістікті түсінудің бірден бір мүмкіндігі олардың функционалдық денгейінің молекулалық негізін зерттеу. Бұл жерде біз Treg жасушаларының адгезияға қабілеті субпопуляцияларның айрмашылығын зертеудің жаңа әдісін көрсеттік. Бұл әдістің көмегімен сау адамдардың шеткі аймақтық қанынан жаңадан бөлініп алынған гиалуранан байланысушы (HA+) CD4+CD25+ жасушалардың сипаттамасын жасадық. Сонымен қатар HA+ CD4+CD25+ субпопуляцияларындағы FoxP3+ және CD39+ жасушаларының саны HA- CD4+CD25+субпопуляцияларынан аз екенін байқадық. Бұл нәтижелер қалыпты айналымдағы табиғи Treg жасушаларының құрамындағы бұл субпопуляцияның жоғары молекулалы гиалурананмен байласуға қабілеттілігін және иммундық аутореактивтілікті басқару мүмкідігіне ие екенін көрсетті.

Түйін сөздер: Т реттегіш жасушалар, гиалуранан , ағынды цитофлуометрия

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Фенотипический анализ cd4+cd25+ трег-клеток периферической крови человека, связывающих и несвязывающих высокомолекулярный гиалуронан

Регуляторные Т-клетки (Трег), как натуральные, так и индуцированные, контролируют разнообразные физиологические и патофизиологические иммунные реакции и обладают высоким терапевтическим потенциалом. Установлено, что популяция Трег-клеток очень гетерогенна, механизм их действия и участие в опухолевом росте, аутоиммунных заболеваниях и в хроническом воспалении не полностью изучены. Одним из ключевых подходов к изучению дефектов функции Трег-клеток при патологических процессах заключается в изучении молекулярной основы их функционального состояния. В настоящей работе мы показали новый подход к исследованию Трег-клеток, который позволяет дискриминировать потенциальную способность Трег-клеток к адгезии на внеклеточном матриксе. Используя этот подход, мы охарактеризовали популяцию свежеизолированных гиалуронансвязывающих (HA+) CD4+CD25+ клеток периферической крови здоровых доноров. Мы показали, что в HA+CD4+CD25+ фракции Т-лимфоцитов содержится меньшее количество FoxP3+CD39+ клеток, чем в HA- фракции. Полученные результаты позволяют предположить, что в норме циркулирующие натуральные Трег-клетки содержат субпопуляцию, способную связывать высокомолекулярный гиалуронан и тем самым контролировать возможные аутоиммунные реакции.

Ключевые слова: Т-регуляторные клетки, гиалуронан, проточная цитометрия.

T regulatory cells can be described as a T cell population that functionally suppresses the immune response by influencing the activity of a range of effector cells and thereby contributes to the maintenance of immune homeostasis. They are crucial for protection against autoimmunity and they also modulate immunity to infections and in tumor [1]. Treg cells originally recognized by their constitutive expression of CD4 and CD25 are further defined by expression of surface CD152 and low expression of CD127. The main marker of Treg cells is Foxp3, a DNA-binding protein, which is necessary for Treg development and function. [2]. Treg cells mediate suppression in cell contact dependent manner. Tregs may kill responder T cells by a granzyme-dependent or perforin dependent mechanism or deliver a negative signal to responder T cells via up-regulating intracellular cyclic AMP, which leads to inhibition of T cell proliferation and IL-2 formation [3]. Additionally, Tregs act by cell-to-cell contact via membrane-bound TGFβ [4], IL-35 and IL-10 [5].

Adhesion of Treg cells and recruitment to sites of inflammation or tumor growth requires binding their CD44 receptor with a component of extracellular matrix – hyaluronan (HA). While highly expressed, CD44 on resting lymphocytes is inactive and binds to HA only when conformationally activated. CD44 can be activated from a low to high HA-binding affinity state on T cells by HA-binding itself, TCR engagement, and responses to cytokines/chemokines [6-8]. Therefore, the ability of Treg cells to interact with HA is intrinsically related to their activation state.

In vitro ligation of CD44 on activated Tregs promotes persistent expression of FoxP3, increased production of IL-10 and also promoted cell surface TGF-β expression, which are necessary for immunoregulatory activity. These functions of CD44 are shown to depend upon interactions with high molecular weight forms of HA that are found in the absence of inflammatory responses. [9].

The aim of our study was to develop a new method of purification of HA+ Treg cells from peripheral blood mononuclear cells (PBMC) using immobilized high molecular weight hyaluronan and to assess their phenotype related to specific suppressor activity.

Materials and Methods

Peripheral blood mononuclear cell isolation. Blood samples (20-30 ml) were obtained from 10 healthy donors. Blood was drawn into sterile tubes with EDTA, mixed well and centrifuged on Histopaque (SigmaAldrich) gradient with density of 1.077 g/mL. PBMC were washed with RPMI 1640 medium (SigmaAldrich), counted in hemacytometer and immediately used for experiments.

Separation of hyaluronan-binding PBMC. 1,86 μg of biotinylated hyaluronan (SigmaAldrich) and 100 μl of MACSiBeads (MiltenyiBiotec) were mixed in 100 μl solution containing phosphate buffer saline (PBS), 0.5% bovine serum albumin (BSA), and 2 mM EDTA and incubated 2 h at 4-8^{o}C under gentle rotation. After that HA-beads were washed in 1ml of buffer at 300 g for 15 min and resuspended in 100 μl of buffer. PBMC suspension was mixed with HA-beads, incubated at 4-8^{o}C for 20 min, then cells were magnetically separated into 2 fractions (HA+ and HA-) using BD IMag Separator.

Antibodies. The following anti-human mAbs were used for flow cytometry: anti-CD4-PerCP, anti-CD25-PE, anti-CD25-FITC, anti-FOXP3-PE, anti-CD39-PE, anti-CD45RO-PE, anti-CD45RA-FITC and anti-IL-10-PE and their relevant control isotypes (BD).

Surface and Intracellular Staining. Cells were incubated with mAbs specific for surface markers (5 μl to 100 μl of cell suspension) for 10 min at 4-8°C in the dark and then fixed and permeabilized (up to 10^7 cells in 500 μl) in Fixation/Permeabilization solution (BD), mixed well and incubate for 20 min in the dark at room temperature. Then cells were centrifuged at 300 g for 15 min. Afterward cells were stained with mAbs specific for intracellular markers (5 μl to 100 μl of cell suspension) for 10 min at 4°C in the dark. Cells were washed with PBS, resuspended in flow solution, and immediately analyzed by flow cytometry. Appropriate isotype controls were included for each sample.

Flow cytometry. FACSCalibur (BD) flow cytometer was used for cell analysis.

Statistical analysis. Data are expressed as median and diapason of 25% and 75% quartiles. Nonparametric Wilcoxon matched pairs signed-rank test was used to determine significance.

Results and Discussion

T regulatory cells of healthy donors can bind hyaluronan. We have not identified any HA-binding cells in freshly isolated CD4+ fraction using soluble biotinylated HA and strepatavidin-FITC conjugate. A. Ariel et al. have shown that approximately 5% of freshly isolated T cells adhere to HA immobilized on plates [8], but this method is not easy-to-use for further analysis of the cells. We have used a new approach to separation of HA-binding cells that provides more quantitative assessment of them. We have isolated HA-binding cells by magnetic separation using biotinylated hyaluronan linked with magnetic anti-biotin particles.

Median HA+ PBMC content in healthy donors was 17.7%. We identified that median of CD4+CD25+ Treg cells was almost equal in both HA+ and HA- fractions of freshly isolated PBMC (12.5% and 14.0%, respectively) (Fig.1).

HA-binding Treg cells distinguish a population with decreased expression of suppressor markers. Definition of Treg cells based on the level of CD25 expression has not been consistenly reported in literature. While CD25 is also expressed on activated T conventional cells, FoxP3 expression has a central role in Treg identification.

As a highly characterized marker of Treg subset, Foxp3 has been shown to be essential for suppressive activity of CD4+CD25+ cells. Prior study (Siegelman M.H. *et al*.) did not identify any visible differences in Foxp3 expression between HA+ and HA- CD4+CD25+ populations after 3 day activation with Anti-CD3 and Anti-CD28 antibodies [10]. We hypothesized that FoxP3+Treg cells circulating in peripheral blood of healthy man permanently contain hyaluronan binding fraction. Indeed, median of FoxP3+CD4+CD25+ T cells was significantly higher in HA- than in HA+ fraction (80% and 26% respectively, $p<0.05$) (Fig. 2A). Interestingly, the opposite tendency was observed in MFI of FoxP3+ that characterized density of marker expression (Fig. 2B).

It has been recently shown that ectonucleotidase CD39 is expressed on various cell types, including Treg cells. Their biologic importance of ectonucleotidase activity is supported by a recent finding that adenosine is one of the major immunosuppressive factors utilized by Treg for regulating tolerance to tissue grafts or cancer and preventing autoimmune diseases [1].

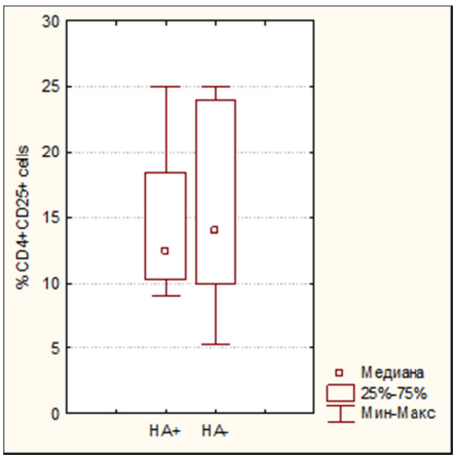
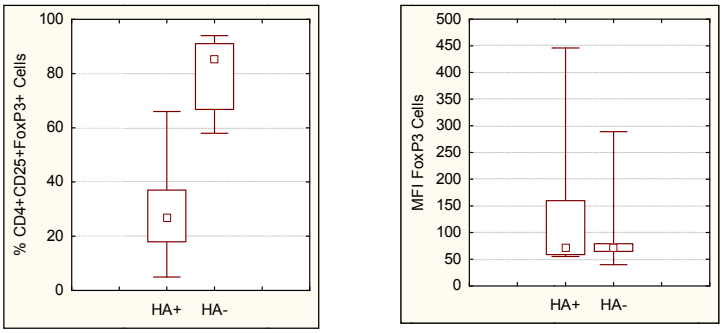
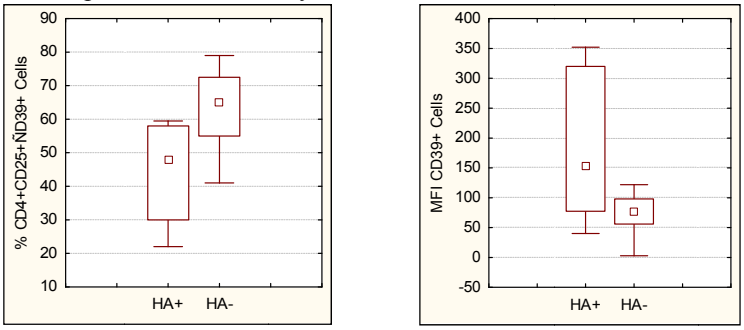


Figure 1 - Hyaluronan-binding and non-binding CD4+CD25+ Treg cells of peripheral blood of healthy donors



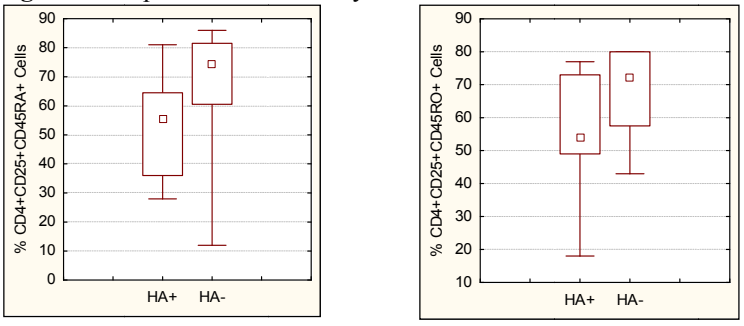
A B

Figure 2 - Expression of FoxP3 by HA+ and HA- subsets of CD4+CD25+ cells



A B

Figure 3 - Expression of CD39 by HA+ and HA- CD4+CD25+ cells



A B

Figure 5 - Expression of CD45RA and CD45RO by HA+ and HA- Treg cells

We have found that median content of CD39+ Treg cells was significantly decreased in HA+ fraction compared with HA- subset ($p<0.05$), though higher density of CD39 expression was observed in HA+ subset ($p<0.05$) (Fig. 3).

We did not identify any significant difference in number in of freshly isolated HA+ and HA- CD4+CD25+ T cells with intracellular expression of IL-10 (0.8% and 1.5% respectively).Since CD4+CD25+ population is known to show increased expression of effector/memory markers (CD45RA/CD45RO), it was of interest to analyze these surface markers on Treg cells with different expression of active isoforms of CD44. As it turned out, there were no differences between HA+ and HA- subsets by these markers (Fig. 5, 6).On the basis of our data we propose that in norm circulating natural Treg cells contain the subset capable of binding high molecular hyaluronan, i.e. expressing an activated form of CD44. Such cells are in a state of readiness to control possible emergence of immune autoreactivity. Though HA+ subset contains less FoxP3+ Treg cells than HA- counterpart CD39 expression on them is higher, that indicates their suppressor potential. Our approach to assessment of Treg cell activity could be useful for studying autoimmune disease and cancer development.

References

1 Sakaguchi S. et al. Regulatory T cell and immune tolerance//Cell.-2008.-N133.-P.775-787.

2 Allan S.E. et al. Generation of potent and stable human CD4+ T regulatory cells by activation- independent expression of FOXP3//Mol. Ther.-2008.-N16.-P. 194-202.

3 Nishikawa H. Sakaguchi S. Regulatory T cells in tumor immunity//Int. J. Cancer.-2010.-N127.-P.759–767.

4 Nakamura K. et al. Cell contact-dependent immunosuppression by CD4+CD25+ regulatory T cells is mediated by cell surface-bound transforming growth factor beta//J. Exp. Med.-2001.-N194.-P.629-644.

5 Vignali D.A., Collison L.W., Workman C.J. How regulatory T cells work//Nat. Rev. Immunol.-2008.-N.8.-P.523-532.

6 DeGrendele H.C. et al. CD44 activation and associated primary adhesion is inducible via T cell receptor stimulation//J. Immunol.-1997.-N.159.-P.2549-2553.

7 Lesley J. et al. Hyaluronan binding function of CD44 is transiently activated on T cells during an in vivo immune response//J.Exp.Med.-1994.-N.180.-P.383-387.

8 Ariel A. et al. Induction of interactions between CD44 and hyaluronic acid by a short exposure of human T cells to diverse pro-inflammatory mediators//Immunology.-2000.-N.100.-P.345-351.

9 Bollyky P.L. et al. CD44 costimulation promotes FoxP3+ regulatory T cell persistence and function via production of IL-2, IL-10, and TGF-β//J.Immunol.-2009.-N.183.-P.2232-2241.

10 Firan M. et al. Suppressor activity and potency among regulatory T cells is discriminated by functionally active CD44//Blood.-2006.-V.107.-N.2.-P.619-627.

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Способы регистрации особенностей экзоцитоза при запуске сигнальной трансдукции

Недостаточно исследованной стадией экзоцитоза является акт слияния гранулярных и плазматических мембран с последующим выбросом содержимого гранул во внеклеточное пространство. Для исследования указанной проблемы были апробированы ряд методических приемов регистрации экзоцитоза на клетках асцитной карциномы Эрлиха (АКЭ) и перитонеальных макрофагах мышей.

Ключевые слова: асцитной карциномы, экзоцитоз, сигнальная трансдукция

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How to register the features of exocytosis at start signal transduction

Insufficiently investigated stage of exocytosis is the act of merging granular and plasma membrane with subsequent release of granule contents into the extracellular space . To investigate this problem have been tried a number of instructional techniques registration exocytosis on cells of Ehrlich ascites carcinoma (EAC) and peritoneal macrophages of mice.

Keywords: ascites , exocytosis , signal transduction

Изучение механизмов экзоцитоза в контексте условий, способствующих малигнизации клеток, является актуальной проблемой онкологии. В связи с чем, изучение связи механизмов экзоцитоза в