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S. PYOGENES M49-16 ARGININE DEIMINASE INHIBITS PROLIFERATIVE ACTIVITY OF HUMAN PERIPHERAL BLOOD LYMPHOCYTES

E.A. Starikova[a,b], T.A. Leveshko[a], D.V. Churakina[b], I.V. Kudryavtsev[a], L.A. Burova[a], I.S. Freidlin[a,b,c]

[a] *Institute of Experimental Medicine, St. Petersburg, Russian Federation*
[b] *Pavlov First St. Petersburg State Medical University, St. Petersburg, Russian Federation*
[c] *St. Petersburg State University, St. Petersburg, Russian Federation*

Abstract. Arginine deiminase is one of three enzymes constituting the arginine deiminase system in bacteria. It was demonstrated that arginine deiminase exerts anti-proliferative effects on some primary and immortalized mouse and human cells. It is assumed that the inhibitory effect of arginine deiminase on cell proliferation might be related to its ability to result in the arginine exhaustion. T-lymphocytes depend on arginine for proliferation, T-cell receptor complex expression, and the differentiation of memory cells. The aim of the current study was to investigate an impact streptococcal arginine deiminase on functions of human peripheral blood lymphocytes. For this, we comparatively analyzed effects of Supernatant of Destroyed Streptococcal Cells (SDSCs) derived from parental strain *S. pyogenes* M49-16 and its isogenic mutant *S. pyogenes* M49-16delArcA bearing inactivated arginine deiminase gene (ArcA) on immune cell functions. An impact of supernatants on cell viability was estimated by staining with DAPI dye. Cell proliferation was assessed by MTT-test and flow cytometry by using the method based on intracellular protein staining with vital fluorescent CFSE (carboxyfluorescein succinimidyl ester) dye. In addition, the level of lymphocyte tyrosine phosphatase CD45 expression in various culturing conditions was evaluated. It was demonstrated that *S. pyogenes* M49-16 SDSCs had no impact on cells viability. Parental strain-derived SDSC exerted virtually no effect on intact cells proliferation, but considerably suppressed ConA-induced cell proliferation. At the same time, mutant strain-derived SDSC significantly stimulated spontaneous cell proliferation, but not that one after mitogen exposure. It was observed that increased proliferation was accompanied by upregulated CD45 expression, although it was not significant in all cases. These data allow to conclude that bacterial arginine deiminase could be one of pathogenicity factors able to limit lymphocyte proliferation and immune response and could be a part of pathogen strategy to suppress immune response in order to improve bacterial growth and dissemination.

Key words: *arginine deiminase, Streptococcus pyogenes, lymphocytes, CFSE, proliferation, CD45.*

АРГИНИНДЕИМИНАЗА *S. PYOGENES* M49-16 ПОДАВЛЯЕТ ПРОЛИФЕРАТИВНУЮ АКТИВНОСТЬ ЛИМФОЦИТОВ ПЕРИФЕРИЧЕСКОЙ КРОВИ ЧЕЛОВЕКА

Старикова Э.А.[1,2], Левешко Т.А.[1], Чуракина Д.В.[2], Кудрявцев И.В.[1], Бурова Л.А.[1], Фрейдлин И.С.[1,2,3]

[1] *ФГБНУ Институт экспериментальной медицины, Санкт-Петербург, Россия*
[2] *Первый Санкт-Петербургский государственный медицинский университет им. акад. И.П. Павлова, Санкт-Петербург, Россия*
[3] *Санкт-Петербургский государственный университет, Санкт-Петербург, Россия*

Резюме. Аргининдеиминаза является одним из трех ферментов, образующих систему аргининдеиминазы у бактерий. Ранее было установлено, что аргининдеиминаза, обладает антипролиферативным действием

Адрес для переписки:
Старикова Элеонора Александровна
197376, Россия, Санкт-Петербург, ул. Академика Павлова, 12,
Институт экспериментальной медицины.
Тел.: 8 (812) 234-16-69 (служебн.). Факс: 8 (812) 234-94-89.
E-mail: Starickova@yandex.ru

Contacts:
Eleonora A. Starikova
197376, Russian Federation, St. Petersburg, Akademika Pavlova str., 12,
Institute of Experimental Medicine.
Phone: +7 (812) 234-16-69 (office). Fax: +7 (812) 234-94-89.
E-mail: Starickova@yandex.ru

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в отношении ряда первичных и иммортализованных клеток мыши и человека. Предполагается, что ингибирующее действие фермента может быть связано с его способностью приводить к истощению аргинина в микроокружении клеток. Биодоступность аргинина играет важную роль для пролиферации Т-лимфоцитов, экспрессии белков Т-клеточного рецептора и дифференцировки клеток памяти. Целью настоящего исследования стало изучение влияния аргининдеиминазы пиогенного стрептококка на функциональную активность лимфоцитов периферической крови человека. Для достижения поставленной цели было проведено сравнительное исследование влияния супернатанта разрушенных стрептококковых клеток (СРС) исходного штамма *S. pyogenes* M49-16 и его изогенного мутанта *S. pyogenes* M49-16delArcA с инактивированным геном аргининдеиминазы (ArcA) на функции иммунных клеток. Влияние супернатантов на жизнеспособность клеток оценивали методом проточной цитометрии, с использованием ДНК-связывающего красителя DAPI. Пролиферацию клеток оценивали с помощью МТТ-теста и цитофлуориметрически с использованием метода, основанного на окрашивании внутриклеточного белка витальным флуоресцентным красителем CFSE (карбоксифлуоресцеинсукцинимидилэфирным красителем). Кроме того, в работе оценивали уровень экспрессии на лимфоцитах тирозинфосфатазы CD45 в различных условиях культивирования. Было показано, что СРС *S. pyogenes* M49-16 не оказывал влияния на жизнеспособность клеток. СРС исходного штамма практически не влиял на пролиферацию интактных клеток, но значительно подавлял пролиферацию клеток, индуцированную ConA. В то же время СРС мутантного штамма достоверно повышал спонтанную пролиферацию клеток и не оказывал влияния на пролиферацию, индуцированную митогеном. Было показано, что увеличение пролиферации сопровождалось увеличением уровня экспрессии CD45, однако эти изменения не во всех случаях были достоверны. Полученные данные позволяют заключить, что бактериальная аргининдеиминаза может являться одним из факторов патогенности, способных ограничивать пролиферацию лимфоцитов и развитие иммунного ответа с целью улучшения роста и диссеминации бактерий.

Ключевые слова: *аргининдеиминаза, Streptococcus pyogenes, лимфоциты, CFSE, пролиферация, CD45.*

Introduction

Arginine deiminase (AD) is one of three enzymes constituting the arginine deiminase system in bacteria [19]. The AD system is widespread among prokaryotes, and for *Mycoplasma* spp. L-arginine catabolism caused by this enzymatic complex serves as the main non-glycolytic energy source [4]. AD system protects bacteria from low pH with ammonia production and improves bacterial growth and biosynthesis processes with citrulline and ATP generation [4, 19]. Initially, streptococcal AD was isolated from *S. hemolyticus* Su strain cell extract in 1985 [33]. Enzyme which at that time was called "streptococci acid glycoprotein (SAGP)" was able to suppress proliferation of transformed hamster embryonic lung cells (THEL) [31]. Later on, the gene encoding this protein was characterized, cloned and expressed in *E. coli* system [17], and only in 1998 it was proved that the product of this gene possesses arginine hydrolyzing activity and has high homology with AD, previously extracted from *Mycoplasma hominis, Mycoplasma arginini, Pseudomonas putida* and *Pseudomonas aeruginosa* [8].

Subsequently, AD activity was also described for other *S. pyogenes* strains [6]. It was demonstrated that *S. pyogenes* AD, as well as homologous enzymes extracted from other microorganisms, displayed antiproliferative effect on a number of primary and immortalized mouse and human cells [7, 29, 30, 32]. Currently, researchers' attention is generally focused on studying mechanisms of anti-tumor impact of this enzyme [18]. Meanwhile, in an adult, immune cells as well as tumor cells show high transient proliferative activity. Clonal expansion and differentiation lymphocytes into effectors and memory cells underlies the adaptive immune response to infection. Arginine bioavailability is an important factor that regulates activation and proliferation of immune cells [23]. Arginine depletion in the site of infection could be a part of pathogen's strategy to suppress immune response in order to improve bacterial growth and dissemination. Thus, the investigation of bacterial AD influence on immune cells function in host organism requires detailed examination to be carried out. The objective of this research is to study *S. pyogenes* M49-16 AD influence on functions of human peripheral blood lymphocytes.

Materials and methods

Obtaining supernatant of ultrasonic destroyed streptococci. S. pyogenes M49-16 delArcA strain was obtained and characterized as described earlier [26]. Supernatant of Destroyed Streptococcal Cells (SDSCs), containing biologically active intracellular components, were extracted from the cultures of parental *S. pyogenes* M49-16 and its isogenic mutant with arcA (AD) gene deletion *S. pyogenes* M49-16 delArcA. Streptococci were cultured within 18–20 hours at 37°C in Todd–Hewitt (Difco) medium in aerobic conditions, sedimented by centrifugation and washed twice with PBS (150 mM NaCl, 10 mM Na-phosphate buffer, pH 7.4), containing no liposaccharide. Concentration of bacterial cells suspension was standardized in accordance with optical density, and its amount was made up to $2.5–5 \times 10^8$ colony-forming units per milliliter. Ultrasonic disintegration of streptococci was carried out with the amount of 5 ml of bacterial suspension in PBS at pH 7.4 for 5 min, at frequency of 22 kHz and power

0.6–0.8 mA on a disintegrator (MSE). The completeness of bacterial cells destruction was controlled microscopically, after that the suspension was centrifuged at 1600g for 30 min. Received supernatants were then sterilized with use of Filtropur S filters with 0.45 μm pore size (Sarstedt, Germany) and stored at –20°C.

Isolation of mononuclear cells from human peripheral blood. Healthy donors' blood of both sexes, aged from 20 up to 50 years old, was collected in a vacuum test tube with K3EDTA. To isolate mononuclear leukocytes, separation was carried out by cells sedimentation on Ficoll solution gradient with 1.077 g/cm^3 density (Biolot, Russia). Blood sample was diluted by 3 times in Hanks's solution (Biolot, Russia), layered on Ficoll solution gradient (Biolot, Russia) and centrifuged in a horizontal rotor at 400g for 40 min. Mononuclear leukocytes were harvested and washed twice in 0.5 mM EDTA solution (Biolot, Russia) at 300g for 15 min at 4°C. Quantity of cells was then counted in Goryaev's chamber.

MTT cell proliferation assay. The impact of SDSCs on PBMCs proliferation was assessed by MTT-test, according to mitochondrial dehydrogenase activity [21]. The cells, isolated as described before, were placed in 96-well plates (Sarstedt, Germany) in concentration of 200 000 cells/100 μl RPMI1640 culture medium (Biolot, Russia) supplemented with 10% of FBS (Invitrogen, USA), 50 μg/ml of gentamycin (Biolot, Russia), 2 mM of glutamine (Biolot, Russia) and 50 μM β-mercaptoethanol (Sigma-Aldrich, USA). For induction of cell proliferation 5 μg/ml ConA (Sigma-Aldrich, USA) was used. SDSCs of parental and mutant strain were added in 1/50 and 1/100 dilution. No SDSCs were added to the control wells. The cells were cultured for 6 days at 37°C in humidified environment with 5% of CO_2.

On the 3rd day of cultivation partial replacement of the cultural medium with the fresh one with supplements was performed. For the last 4 hours of experiment MTT was added to each well (Sigma-Aldrich, USA) in final concentration of 0.5 mg/ml. After that 100 μl of lysing buffer, containing 10% sodium dodecyl sulfate (Serva Electrophoresis, Germany) in 0.01N-HCl was added, and incubated at 37°C over night. Results were analyzed by spectrophotometer (Microplate reader, model 680, BioRad, USA) at the wavelength of 570 nm. Proliferation level was estimated according to optical density in wells. The results were shown in percentage. Optical density in the control wells, containing cultural medium was defined as 100%.

Cell proliferation assay by flow cytometry. For proliferation assessment we used a method based on intracellular protein staining by vital fluorescent CFSE (carboxyfluorescein succinimidyl ester) dye (Sigma-Aldrich, USA). Isolated, as described before, PBMCs were brought to concentration 1×10^6 cells per ml of Hanks solution (Biolot, Russia) with CFSE (Sigma-Aldrich, USA) in final concentration of 0.5 μg/ml and left for 10 min in water bath, at 37°C. After that the cells were washed twice by centrifugation at 300g, 4°C, for 15 min in cold Hanks solution containing 1% of FBS (Sigma-Aldrich, USA). CFSE stained PBMCs were placed in 24-well plate in concentration of 2 000 000 cells/ml RPMI 1640 culture medium (Biolot, Russia) containing 10% of FBS (Invitrogen, USA), 50 μg/ml of gentamycin (Biolot, Russia), 2 mM of glutamine (Biolot, Russia) and 50 μM β-mercaptoethanol (Sigma-Aldrich, USA). ConA at final concentration of 5 μg/ml was used for proliferation induction. SDSCs of parental and mutant strains were added in 1/50 and 1/100 dilution. No SDSCs were added to the control wells. On the 3rd day of cultivation partial replacement of the cultural medium with the fresh one with supplements was carried out. After 6-day cultivation the cells were harvested in cytometry test tubes (Beckman Coulter, USA) and stained with PC5 conjugated CD45 antibodies (Beckman Coulter, USA). For necrosis level assessment cellular suspension was stained with 300 nM of DNA-binding dye DAPI (Invitrogen, USA). Samples assessment was carried out by a flow cytometer Navios™ (Beckman Coulter, USA).

Statistical analysis. The differences between experimental group were tested for significance by Student's t-test for independent group. In all tests zero hypothesis was rejected at $p > 0.05$. Analysis were carried out using the Statistica 8.0 (StatSoft, USA).

Results

The influence of SDSCs on PBMCs proliferation (Table 1). Initially, cells were gated on the living (DAPI-negative) and CD45^{high} (lymphocyte). It was demonstrated that under standard conditions of cultivation the proportion of lymphocytes which did not undergo any divisions made up to 99% (Table 2, Fig., bottom row). Parental strain SDSCs had an effect only in dilution 1/100, expressed in a significant increase in the proportion of cells experiencing 1 division. Incubation in presence of mutant strain SDSCs resulted in a significant increase in the persentages of cells undergoing up to 7 and 8 divisions with SDSCs 1/50 and 1/100 dilutions, respectively. Meanwhile, a significant decrease in the proportion of non-divided cells to 65% with maximum SDSC dilution was observed.

In case of ConA treatment the proportion of cells which underwent up to 8 divisions increased; simultaneously, the proportion of non-divided cells decreased up to 43% (Table 3, Fig., bottom row). Parental strain SDSC significantly suppressed ConA-induced proliferation that was expressed in doubling in the proportion of non-divided cells with a decrease in the proportion of divided ones. The strongest inhibiting effect of parental strain SDSC was observed in the maximum concentration (dilution 1/50). In contrast,

Table 1. The influence of SDSCs on PBMCs viability

	Proportion of live DAPI$^-$CD45^{hi} cells (%, M±m, n = 5), after incubation				
	without SDSCs	with SDSC M49-19		with SDSC M49-16delArcA	
		1/50	1/100	1/50	1/100
Culture medium (control)	71.3±9.03	71.6±5.32	71.6±4.36	71.4±4.31	74.2±3.36
ConA 5 μg/ml	74.7±14.16	63.1±10.20*	62.9±11.99	62.1±26.27	70.6±24.58

Notes. * — statistically significant difference compared to the control ($p < 0.05$). A significant decrease in living cells proportion took place only in the presence of ConA and parental strain SDSC in dilution 1/50 and made up 10%. It is noteworthy that ConA and SDSCs of *S. pyogenes* M49-16 had no independent impact on cells viability.

Table 2. The influence of SDSCs on the human peripheral blood lymphocyte spontaneous proliferation

Number of divisions	Proportion of CD45^{hi} cells (M±m) which underwent corresponding number of divisions, after incubation with				
	Culture medium (control)	SDSC M49-19		SDSC M49-16delArcA	
		1/50	1/100	1/50	1/100
0	98.7±0.3	91.3±4.5	85.3±6.8	65.3±6.4*	70.0±2.5*
1	1.2±0.31	3.3±1.12	4.5±1.31*	5.5±0.95*	5.1±0.88*
2	0.1±0.02	1.9±1.09	3.1±1.45	5.0±0.78*	4.7±0.61*
3	0.04±0.024	1.4±0.94	2.4±1.33	5.2±0.61**	4.9±0.42*
4	0.02±0.020	1.0±0.68	1.9±1.16	5.2±0.85**	4.8±0.41**
5	0	0.9±0.61	1.8±1.03	5.2±1.13**	4.3±0.18**
6	0	0.2±0.24	0.8±0.50	3.8±0.94*	3.2±0.33**
7	0	0.1±0.10	0.4±0.24	2.3±0.74*	1.9±0.31**
8	0	0	0.2±0.14	2.2±1.38	0.9±0.31**

Notes. * — statistically significant difference compared to the control; ** — statistically significant difference compared to the SDSC *S. pyogenes* M49-16 in corresponding dilution ($p < 0.01$).

mutant strain SDSC showed a weak inhibiting effect on ConA-induced lymphocytes proliferation. In this case the proportion of cells which underwent up to 4 divisions slightly decreased in presence of SDSC dilution 1/50.

Assessment of PBMCs proliferative activity was carried out with use of MTT-test. Parental strain SDSC had no impact on intact cells proliferation, but suppressed the proliferation induced by ConA (Table 4). Mutant strain SDSC significantly stimulated spontaneous cell proliferation and had no significant impact on mitogen-induced proliferation.

The influence of SDSCs on CD45 tyrosine phosphatase expression. The mutant strain SDSC significantly enhanced CD45 expression on non-divided cells given that they were not stimulated by ConA. Herewith on dividing cells, CD45 expression significantly increased. PBMCs incubation in the pres-

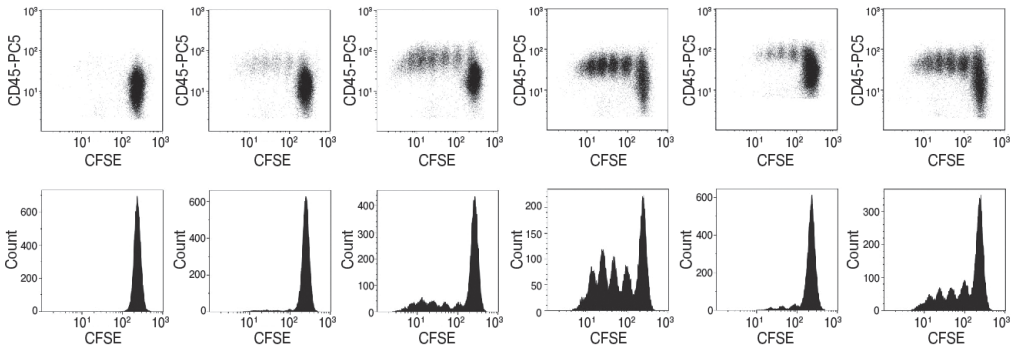

Figure. Effect of SDSCs on the human peripheral blood lymphocyte proliferation and CD45 expression
Notes. Upper row. Representative dot plots show CD45 expression versus CFCE fluorescence. From left to right: culture media, M49-16, M49-16delArcA, ConA, ConA and M49-16, ConA and M49-16delArcA. Bottom row. Representative histograms show CFCE fluorescence. From left to right: culture media, M49-16, M49-16delArcA, ConA, ConA and M49-16, ConA and M49-16delArcA.

Table 3. The influence of SDSCs on the human peripheral blood lymphocyte proliferation induced by ConA

Number of divisions	Proportion of cells (%, M±m) which underwent corresponding number of divisions, cultivated with				
	ConA (control)	SDSC M49-19		SDSC M49-16delArcA	
		1/50	1/100	1/50	1/100
0	42.7±3.8	81.6±9.8*	69.6±9.9*	47.6±6.6$	47.4±6.3
1	10.4±1.1	6.4±2.35	9.8±1.90	10.1±0.72	10.6±0.80
2	9.3±1.13	4.0±2.25	6.1±2.47	9.0±0.55	9.4±0.76
3	9.2±0.98	3.2±1.98*	6.0±2.23	8.4±0.65**	8.8±0.80
4	9.3±1.16	2.4±1.55*	4.7±1.88	7.5±0.95**	8.0±1.08
5	8.3±1.29	1.5±1.06*	2.4±1.31*	7.0±1.51**	7.3±1.53**
6	5.5±0.71	0.7±0.52*	1.1±0.60*	5.3±1.52**	5.1±1.30**
7	3.0±1.03	0.3±0.26*	0.3±0.27*	3.1±1.00**	2.4±0.42**
8	1.4±0.86	0	0.02±0.020	1.4±0.64	1.0±0.26**

Notes. * — statistically significant difference compared to the control; ** — statistically significant difference compared to the SDSC *S. pyogenes* M49-16 in corresponding dilution ($p < 0.01$).

ence of parental and mutant strains SDSCs caused a significant increase in CD45 expression on dividing cells not stimulated by mitogen, compared with control. But in this case, there was no significant difference in the effects showed by these two SDSC strains.

Under the influence of ConA, the expression of CD45 significantly increased on both non-divided and divided cells in comparison with the corresponding controls. The difference in the marker expression on divided and on non-divided cells was significant only for the parental strain SDSC. For the mutant strain SDSC, there was no significant difference between these parameters. (Tabl. 5, Fig., upper row).

Discussion

In current work the influence of streptococcal AD on functional activity of human peripheral blood lymphocyte was carried out. The study was carried out with *S. pyogenes* M49-16 isogenic mutant lacking the ability to make AD that has been constructed and characterized earlier [27]. An assay SDSCs prepared from the parental and mutant strains showed that significant AD activity was present in parental SDSC but none could be detected in mutant one [26]. It was established that parental and mutant strains SDSC did not exhibit toxic impact on human PBMCs (Tabl. 1). Cell death was observed to increase only in case of concomitant

Table 4. The influence of SDSCs on the PBMCs proliferation

	Total proliferation level of PBMCs (%, M±m, N = 14) cultivated				
	without SDSCs	with SDSC M49-19		with SDSC M49-16delArcA	
		1/50	1/100	1/50	1/100
Cultural medium (control)	100	87.4±9.32	93.0±11.28	147.2±13.77**	143.3±13.04**
Con A 5 mkg/ml	133.6±14.16*	101.3±10.20	106.3±11.99	174.4±26.27**	163.2±24.58**

Notes. * — statistically significant difference compared to the control; ** — statistically significant difference compared to the SDSC *S. pyogenes* M49-16 in corresponding dilution ($p < 0.01$).

Table 5. SDSCs impact on CD45 expression on PBMCs

Cell culturing in presence	The level of CD45 expression, the mean fluorescence intensity (MFI, M±m, n = 4)			
	without ConA		with ConA	
	non-dividing cells	dividing cells	non-dividing cells	dividing cells
Culture media (control)	13.5±3.64	21.7±2.48***	25.9±1.16**	40.5±7.45**
M49-19 1/50	17.5±1.16	30.9±1.81***	20.7±1.77	34.7±3.29***
M49-19 1/100	18.1±0.83	35.8±5.14***	19.9±2.84	37.0±4.72***
M49-16delArcA1/50	20.8±5.82*	37.5±3.31***	31.5±1.67	47.5±13.02
M49-16delArcA1/100	23.4±4.83	40.2±6.27***	28.1±4.77	40.6±9.47

Notes. * — expression level is significantly different from control ($p < 0.05$); ** — expression level is significantly lower than expression level with ConA; *** — expression level with SDS *S. pyogenes* M49-16 is significantly lower than expression level with SDS *S. pyogenes* M49-16 delArcA in corresponding dilution.

cells cultivation in presence of ConA and parental strain SDSC. Parental and mutant strains SDSCs had an opposite effect on the proliferation of untreated and ConA-stimulated cells. Parental strain SDSC slightly increased the level of spontaneous cells proliferation (Tabl. 2, Fig., bottom row) and, on the contrary, significantly suppressed proliferation induced by ConA (Tabl. 3, Fig., bottom row). Meanwhile, mutant strain SDSC had a significant stimulating effect on lymphocytes spontaneous proliferation (Tabl. 2, Fig., bottom row), but showed no effect on ConA-induced lymphocyte proliferation (Tabl. 3, Fig., bottom row).

Comparison of the effects of parental and mutant strains SDSC which differ by only one protein expression showed that AD is the factor which inhibits lymphocytes proliferation. These results confirm the data of other researchers demonstrating a decrease in proliferative activity of human PBMCs under the influence of AD obtained from commensal bacteria of oral cavity *Granulicatella elegans* [16]and pathogenic bacterium of *S. pyogenes* Manfredo strain [8].

It is assumed that the inhibitory effect of AD on lymphocytes proliferation could be caused by the enzyme's ability to result in the arginine exhaustion in the culture medium. There is a lot of data proving that arginine is necessary for lymphocytes proliferation, T-cell receptor expression and memory cells differentiation [3, 11, 23]. Arginine depletion is the central pathway that was used by arginase-expressing myeloid suppressor cells to limit effector T-cell functions [9, 10, 20, 24]. It is shown that myeloid suppressor cells cause inhibition of T lymphocytes proliferation in vitro due to decreased expression of T-cell receptor CD3ζ chain [22]. Arginine is the substrate for both enzymes — arginase and AD, but metabolites of these enzymes are quite different. Unlike arginase, which converts arginine into ornithine, bacterial AD catalyzes arginine conversion in citrulline and the deficiency of arginine can be compensated by the re-synthesis of this amino acid from citrulline. It was shown that the proliferation of activated T lymphocytes reduced due to arginine deprivation *in vitro* could be restored with citrulline addition [2]. Besides, in the conditions of low arginine concentration an amino-acid transpoter CAT-1 expression and citrulline transport up-regulated in T lymphocytes [15]. In addition, argininosuccinate synthase expression increased providing citrulline conversion into arginine [2, 9, 28]. Therefore, the mechanisms of AD influence on the functional activity of lymphocytes may have their own features in comparison with the mechanisms defining the action of arginase.

In the current research it was discovered that parental and mutant strains SDSCs had an opposite effect on the proliferation of unstimulated and mitogen-stimulated cells. SDSCs comprise the factors, which stimulate lymphocytes proliferation. It is well known that *S. pyogenes* express a wide range of factors, which could act as super antigens, i. e. induce lymphocytes polyclonal activation. Among them are streptococcal pyrogenic exotoxins (SPEs) [25]. The effect of these substances was manifested when SDSC from the mutant strain, lacking AD, was added to PBMCs culture. However, AD could completely grade the mitogenic effect of these factors, since the parental strain SDSC, containing AD, exhibit no proliferating activity. It is probable that the presence of factors with opposite actions could allow a pathogen to adapt to the environmental conditions changing during infection. For example, SpeB plays an important role in early stages of local streptococcal infections [1, 12, 13]. Opposite, arginine depletion could be manifested later, when the bacterial load and AD output in the site of pathogen invasion dramatically increase. In our previous research, using murine model of streptococcal infection we found a gradual arginine decrease in blood showing the lowest values on the fifth day of post-infection [26].

In this work it was established that lymphocyte proliferative activity was correlated with CD45 tyrosine phosphatase expression level (Tabl. 5, Fig., upper row). It is known that CD45 regulates various cell processes, including mitotic cycle, cell growth and differentiation. Intracellular domain CD45 possesses protein tyrosine phosphatase activity and plays an important role in signal transduction from T-cell receptor. CD45 activates Lck tyrosine phosphatase by removal of inhibiting phosphate group from tyrosine [5]. It was established that CD45 regulates immune cells functions, changing their sensitivity to variety of activation factors [14]. Based on the obtained data it is impossible to claim for certain whether CD45 reduced expression caused cells proliferation inhibition or vice versa. It is quite possible that there is a positive regulatory relation between cells proliferative activity and CD45 expression.

In conclusion, due to inhibition of lymphocyte proliferation, bacterial AD could be one of pathogenicity factors that is able to limit immune cells activation. It is likely to restrict immune response to commensal microbiota but also be a part of pathogen's strategy directed to suppress adaptive immune response during infection.

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Авторы:

Старикова Э.А., к.б.н., старший научный сотрудник отдела иммунологии ФГБНУ Институт экспериментальной медицины, Санкт-Петербург, Россия; доцент кафедры иммунологии и ГБОУ ВПО Первый Санкт-Петербургский Государственный медицинский университет им. акад. И.П. Павлова МЗ РФ, Санкт-Петербург, Россия;
Левешко Т.А., лаборант отела иммунологии ФГБНУ Институт экспериментальной медицины, Санкт-Петербург, Россия;
Чуракина Д.В., студент ГБОУ ВПО Первый Санкт-Петербургский Государственный медицинский университет им. акад. И.П. Павлова МЗ РФ, Санкт-Петербург, Россия;
Кудрявцев И.В., к.б.н., старший научный сотрудник отдела иммунологии ФГБНУ Институт экспериментальной медицины, Санкт-Петербург, Россия;
Бурова Л.А., д.м.н., ведущий научный сотрудник отдела молекулярной микробиологии ФГБНУ Институт экспериментальной медицины, Санкт-Петербург, Россия;
Фрейдлин И.С., д.м.н., профессор, член-корреспондент РАН, главный научный сотрудник отдела иммунологии ФГБНУ Институт экспериментальной медицины, Санкт-Петербург, Россия; профессор кафедры иммунологии ГБОУ ВПО Первый Санкт-Петербургский Государственный медицинский университет им. акад. И.П. Павлова МЗ РФ; профессор кафедры фундаментальных проблем медицины и медицинских технологий ФГБОУ ВО Санкт-Петербургский государственный университет, Санкт-Петербург, Россия.

Authors:

Starikova E.A., PhD (Biology), Senior Researcher, Department of Immunology, Institute of Experimental Medicine, St. Petersburg, Russian Federation; Associate Professor, Department of Immunology, Pavlov First St. Petersburg State Medical University, St. Petersburg, Russian Federation;
Leveshko T.A., Technician, Department of Immunology, Institute of Experimental Medicine, St. Petersburg, Russian Federation;
Churakina D.V., Student, Pavlov First St. Petersburg State Medical University, St. Petersburg, Russian Federation;
Kudryavtsev I.V., PhD (Biology), Senior Researcher, Department of Immunology, Institute of Experimental Medicine, St. Petersburg, Russian Federation;
Burova L.A., PhD, MD (Medicine), Leading Researcher, Department of Molecular Microbiology, Institute of Experimental Medicine, St. Petersburg, Russian Federation;
Freidlin I.S., PhD, MD (Medicine), Professor, RAS Corresponding Member, Head Researcher, Department of Immunology, Institute of Experimental Medicine, St. Petersburg, Russian Federation; Professor of the Department of Immunology, Pavlov First St. Petersburg State Medical University, St. Petersburg, Russian Federation; Professor of the Department of Fundamental Problems of Medicine and Medical Technologies, St. Petersburg State University, St. Petersburg, Russian Federation.

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