

**DETERMINING PARTICIPATION PERIPHERAL BLOOD B-CELLS,  
B-REG CELLS, AND MEMORY B-REG CELLS IN DIABETES  
MELLITUS TYPE I DISEASE AND THE EFFECT OF THE URINARY  
TRACT INFECTION RESPONSE**

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**ОПРЕДЕЛЕНИЕ РОЛИ ПЕРИФЕРИЧЕСКИХ В-КЛЕТОК КРОВИ, В-РЕГУЛЯТОРНЫХ КЛЕТОК И В-РЕГУЛЯТОРНЫХ КЛЕТОК ПАМЯТИ ПРИ САХАРНОМ ДИАБЕТЕ 1 ТИПА И ВЛИЯНИЕ ОТВЕТА НА ИНФЕКЦИЮ МОЧЕВЫВОДЯЩИХ ПУТЕЙ**

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## Abstract

**Background.** Type 1 diabetes mellitus (T1DM) is a complex autoimmune disorder. Despite the crucial anti-inflammatory role of IL-10-secreting memory regulatory B cells (memory Bregs), their specific profile and function in T1DM remain poorly understood. The aim of the study was to investigate the peripheral blood frequencies of total B cells, regulatory B cells (Bregs), and memory Breg cells (CD19<sup>+</sup>IL-10<sup>+</sup> CD24<sup>hi</sup> CD27<sup>+</sup>) in patients with Type 1 Diabetes Mellitus (T1DM). Furthermore, the study aimed to evaluate the impact of concurrent urinary tract infections (UTIs) on these immune profiles by comparing T1DM patients with and without UTIs to healthy controls, thereby elucidating the interplay between bacterial infections, Breg cell depletion, and T1DM pathogenesis. **Materials and methods.** This case-control study evaluated male children T1DM patients, categorized by the presence or absence of concurrent UTIs, alongside a healthy control group. Flow cytometry was utilized to quantify the frequencies of total B cells and memory Bregs subsets. Additionally, clinical markers including glutamic acid decarboxylase autoantibodies (GADA) and C-peptide levels were assessed. **Results.** A significant decrease in total B cells and overall regulatory B cells was observed among T1DM patients, particularly those with concurrent UTIs. Notably, memory Bregs exhibited a significant stepwise decline: dropping from 47.07% in healthy controls to 26.97% in T1DM patients without UTIs, and further decreasing to 21.98% in those with UTIs. Coupled with elevated GADA and diminished C-peptide levels, these findings demonstrate that bacterial infections significantly exacerbate the depletion of regulatory B cell subsets. **Conclusion.** The profound depletion of total B, Breg, and memory Breg cells in T1DM children exacerbated by concurrent bacterial infections critically drives the loss of immune tolerance and  $\beta$ -cell destruction. Therefore, restoring memory Breg function offers a promising immunotherapeutic strategy for T1DM.

**Keywords:** B-cells, regulatory B-cells, memory B-cells, Type 1 Diabetes mellitus, urinary tract infection, immune response.

## Резюме

**Введение.** Сахарный диабет 1 типа (СД1) — комплексное аутоиммунное заболевание. Несмотря на решающую противовоспалительную роль (Breg-клеток), секретирующих ИЛ-10, их специфический профиль и функция при СД1 изученными остаются недостаточно. Целью исследования было изучение частоты определения в периферической крови общих В-клеток, регуляторных В-клеток (Breg) и Breg-клеток памяти (CD19+IL-10+ CD24hi CD27+) у пациентов с сахарным диабетом 1 типа (СД1). Кроме того, также оценивалось влияние сопутствующих инфекций мочевыводящих путей (ИМВП) на иммунные профили указанных популяций В-клеток при их сравнении у пациентов с СД1 с ИМВП и без них со волонтерами контрольной группы для установления взаимосвязи между бактериальными инфекциями, истощением Breg-клеток и патогенезом СД1. **Материалы и методы.** В исследовании типа «случай-контроль» оценивались мальчики с СД1, разделенные по наличию или отсутствию сопутствующих ИМВП, наряду со здоровой контрольной группой. Для количественной оценки частоты встречаемости общих В-клеток и субпопуляций регуляторных В-клеток памяти использовалась проточная цитометрия. Кроме того, были оценены клинические маркеры, включая аутоантитела к глутаминовой декарбоксилазе (GADA) и уровни С-пептида. **Результаты.** У пациентов с СД1, особенно при сопутствующих ИМВП, наблюдалось значительное снижение общего количества В-клеток и регуляторных В-клеток в целом. Примечательно, что количество Breg-клеток памяти демонстрировало достоверное ступенчатое снижение: с 47,07% у здоровых контрольных лиц до 26,97% у пациентов с СД1 без ИМВП и далее до 21,98% у лиц с ИМВП. В сочетании с повышенным уровнем GADA и сниженным уровнем С-пептида полученные данные показывают, что бактериальные инфекции значительно усиливают истощение субпопуляций регуляторных В-клеток. **Заключение.** Выраженное снижение общего

количества В-клеток, регуляторных В-клеток и регуляторных В-клеток памяти у детей с СД1, усугубляемое сопутствующими бактериальными инфекциями, критически влияет на потерю иммунной толерантности и разрушение  $\beta$ -клеток. Таким образом, восстановление функции регуляторных В-клеток памяти представляет собой многообещающую иммунотерапевтическую стратегию для лечения сахарного диабета 1 типа.

**Ключевые слова:** В-клетки, регуляторные В-клетки, В-клетки памяти, сахарный диабет 1 типа, инфекция мочевыводящих путей, иммунный ответ.

## 1 **1 Introduction**

2       Type 1 diabetes mellitus (T1DM) is a chronic autoimmune disease  
3 characterized by the progressive, immune-mediated destruction of insulin-producing  
4 pancreatic beta ( $\beta$ ) cells within the islets of Langerhans [15,24]. This destruction  
5 leads to an absolute insulin deficiency and profoundly impaired glucose  
6 homeostasis [51]. The pathogenesis of T1DM is marked by significant alterations  
7 across multiple lymphocyte populations [42]. While autoreactive CD4<sup>+</sup> and CD8<sup>+</sup>T  
8 cells serve as the primary effectors of  $\beta$ -cell destruction, defects in regulatory T cells  
9 (Tregs) also heavily contribute to the loss of peripheral immunological tolerance  
10 [52]. Clinically, the first stage of T1DM is identified by the presence of  
11 autoantibodies targeting pancreatic  $\beta$ -cell antigens, such as GAD [1], zinc  
12 transporter 8 (ZnT8) [45], or islet antigen 2 (IA-2) [16]. The presence of these  
13 autoantibodies, coupled with declining C-peptide levels, underscores the critical and  
14 complex involvement of B-lymphocyte populations in the initiation and progression  
15 of the disease [23]. Beyond their traditional role in autoantibody production, specific  
16 B cell subsets, known as regulatory B cells, play an essential immunosuppressive  
17 role [29]. Originating from bone marrow precursors, Breg cells differentiate in the  
18 periphery in response to specific inflammatory signals and microenvironmental cues  
19 [49]. They are instrumental in maintaining peripheral tolerance and restraining  
20 chronic inflammation in various pathologies, including rheumatoid arthritis [2],  
21 celiac disease [3], and experimental autoimmune encephalomyelitis [20]. In humans,  
22 immature transitional CD19<sup>+</sup>CD24<sup>hi</sup> CD38<sup>hi</sup> B cells can acquire regulatory  
23 capacity following CD40 stimulation. They partially suppress T helper cell  
24 differentiation primarily through the secretion of interleukin-10 (IL-10) [47], and  
25 their inhibitory capacity is further enhanced in the presence of CD80 ligation [21].  
26 However, the dysfunction of these cells is a hallmark of several autoimmune  
27 pathologies. For instance, CD19<sup>+</sup> CD24<sup>hi</sup> CD38<sup>hi</sup> B cells isolated from patients  
28 with systemic lupus erythematosus (SLE) exhibit resistance to further CD154

29 stimulation, secrete significantly less IL-10, and lack the suppressive capabilities  
30 characteristic of healthy B cells [44]. Despite this growing evidence, the precise role  
31 of Breg cells in maintaining peripheral tolerance in human T1DM remains  
32 insufficiently defined. Recent immunological advances have highlighted the  
33 existence of a highly specialized subset known as memory Bregs. These cells  
34 uniquely merge the long-term survival characteristics of memory B cells with the  
35 potent suppressive functions of regulatory cells [27,31]. Phenotypically  
36 characterized as CD19<sup>+</sup> IL-10<sup>+</sup>CD24<sup>hi</sup>CD27<sup>+</sup> cells, memory Bregs are capable of  
37 mounting a rapid, robust, and highly effective regulatory response upon re-exposure  
38 to an antigen [22,32]. They actively participate in inhibiting T cell activation,  
39 reducing the overall production of pro-inflammatory cytokines, and facilitating the  
40 restoration of immune homeostasis in severely inflammatory environments [19].  
41 The progression of T1DM is closely intertwined with environmental triggers,  
42 particularly bacterial infections. Chronic infections can exacerbate systemic  
43 inflammation, thereby increasing insulin resistance and negatively affecting  
44 glycemic control [40]. Conversely, persistent hyperglycemia in T1DM significantly  
45 increases susceptibility to recurrent bacterial infections, particularly UTIs [35]. We  
46 specifically focused on UTIs because diabetes-induced glucosuria creates an ideal  
47 metabolic environment for bacterial colonization, a condition frequently exacerbated  
48 by autonomic neuropathy affecting bladder function [10]. Consequently, UTIs serve  
49 as a frequent, clinically significant inflammatory stressor that can profoundly impact  
50 the immune landscape of diabetic patients [18,33]. Understanding how frequent  
51 bacterial infections, such as UTIs, influence the numerical and functional profiles of  
52 specific immune cells is essential for developing effective preventive and  
53 therapeutic strategies. Therefore, the objective of this study was to investigate the  
54 peripheral blood frequencies of total B cells, regulatory B cells (Bregs), and  
55 specifically memory Breg cells (CD19<sup>+</sup>IL-10<sup>+</sup>CD24<sup>hi</sup> CD27<sup>+</sup>) in patients with  
56 T1DM. Furthermore, the study aimed to evaluate the direct impact of concurrent  
57 UTIs on these immune cell profiles by comparing T1DM patients with and without

58 UTIs to healthy controls. Ultimately, this approach seeks to elucidate the interplay  
59 between bacterial infections, the depletion of regulatory B cell subsets, and the  
60 pathogenesis of T1DM.

## 61 **2 Materials and methods**

### 62 *Patients and controls*

63 The sample size comprised 90 male children participants aged between 5 and  
64 15 years. This included 60 children diagnosed with T1DM (categorized into 30  
65 patients with UTIs and 30 without UTIs) and 30 healthy children serving as a control  
66 group. The clinical component of the study was conducted at Al-Hussein Teaching  
67 Hospital in Karbala. The diagnosis of T1DM was established based on the World  
68 Health Organization (WHO) criteria, specifically a typical history of elevated fasting  
69 blood glucose (FBG) levels and Hemoglobin A1c (HbA<sub>1c</sub>), accompanied by a  
70 decrease in C-peptide secretion in patients exhibiting at least one pancreatic  
71 autoantibody, such as GADA [5]. The mean duration of the disease for patients with  
72 T1DM was (4.2 ± 1.1) years. The healthy control group had no history of malignancy  
73 or autoimmune diseases, and none of the participants were receiving  
74 immunosuppressive medications. Furthermore, all participants were confirmed to be  
75 free of allergies or other chronic infectious diseases .

### 76 *Blood collection and processing*

77 Six milliliters of peripheral venous blood were drawn from each subject and  
78 distributed into two types of tubes: an ethylenediaminetetraacetic acid (EDTA) tube  
79 for flow cytometry and HbA<sub>1c</sub> analysis, and a gel tube for GADA and C-peptide  
80 secretion assays. **For flow cytometric analysis, peripheral blood mononuclear  
81 cells (PBMCs) were subsequently isolated from the EDTA-treated blood using  
82 density gradient centrifugation[13].**

83 *Clinical parameters tests*

84 Fasting blood glucose levels, connecting peptide (C-peptide), and HbA<sub>1c</sub> were  
85 measured using the cobas e 411 analyzer (**Roche Diagnostics, Mannheim,**  
86 **Germany**). GADA were quantitatively estimated using commercial Enzyme-  
87 Linked Immunosorbent Assay (ELISA) kits (**Elabscience, Wuhan, China**)  
88 according to the manufacturer's instructions [11].

89 *Measurement of immunological markers by flow cytometry*

90 The frequencies of total B cells (CD19+), regulatory B cells (CD19+ IL-10+  
91 Breg cells), and memory Bregs (CD19+ IL-10+ CD24<sup>hi</sup> CD27+ memory Bregs)  
92 were determined using flow cytometry. The tests were performed at Imam Zain Al-  
93 Abidin Hospital using a flow cytometer (BD Biosciences, San Jose, CA, USA). The  
94 human monoclonal antibodies utilized for surface and intracellular staining  
95 included: anti-IL-10 APC-R700 (clone JES3-19F1), anti-CD19 PE-CY7 (clone  
96 HIB19), anti-CD24 FITC (clone ML5), and anti-CD27 PE (clone L128) (all  
97 purchased from BD Biosciences, San Jose, CA, USA). To accurately establish gating  
98 boundaries and exclude non-specific binding, appropriate fluorochrome-conjugated  
99 isotype control antibodies (BD Biosciences) were utilized during the optimization  
100 phase. For the detection of surface markers, the isolated PBMCs were stained with  
101 the respective monoclonal antibodies and incubated in the dark at room temperature  
102 to prevent photobleaching of the fluorochromes. To detect intracellular IL-10  
103 expression within the CD19+B cell population, it was necessary to prevent cytokine  
104 secretion by inhibiting intracellular protein transport. Therefore, the BD IntraSure™  
105 Kit (containing Intra A and Intra B reagents) was employed. This kit provides ready-  
106 to-use permeabilization reagents that allow for optimal intracellular staining while  
107 preserving the integrity of cell surface markers [32,38]. The samples were analyzed  
108 utilizing a specific gating strategy, as detailed in Figure 1.

109 *Urine culture and diagnosis of urinary tract infection*

110 Urine samples (10 mL) were collected from all study participants to identify  
111 potential bacterial causes of UTIs. The samples were cultured aerobically on  
112 MacConkey agar and Blood agar at 37°C for 48 hours [32]. The primary  
113 classification of patients into the UTI group was based on quantitative colony  
114 counting rather than extensive species differentiation. After the incubation period,  
115 the number of bacterial colonies was counted to determine the bacterial  
116 concentration. Samples were considered positive for a UTI if the plates yielded  $\geq 10^5$   
117 colony-forming units (CFU)/mL. Samples with fewer than were considered negative  
118 and were excluded from the UTI categorization [37].

119 *Statistical analysis*

120 Data analysis was performed using the Statistical Package for the Social  
121 Sciences (SPSS) software, version 25 (IBM Corp., Armonk, NY, USA). Analysis  
122 of Variance (ANOVA) with a 95% confidence interval was utilized to assess the  
123 statistical differences between the study groups. A  $p$ -value of  $\leq 0.05$  was considered  
124 statistically significant. All results are expressed as the mean  $\pm$  standard error of the  
125 mean (SEM) [43].

126 *Ethical approval*

127 This study was conducted in accordance with the ethical principles set  
128 forth in the Declaration of Helsinki. Researchers obtained both oral and written  
129 informed consent from all participants before sample collection. The study protocol,  
130 participant information, and consent form were reviewed and formally approved by  
131 the local ethics committee, as per document number 240 dated 14/02/2022.

132 **3 Results**

133 *Clinical and demographic characteristics of participants*

134 The study included a total of 90 male children as participants, equally  
135 distributed into three distinct groups: children with T1DM and concurrent UTIs  
136 (n=30), children with T1DM without UTIs (n=30), and a healthy control group  
137 (n=30). The detailed clinical and demographic characteristics of all participants are  
138 summarized in **Table 1**.

139 As expected, a highly significant increase in FBG and HbA<sub>1c</sub> levels was  
140 observed in both diabetic groups compared to the healthy controls. Furthermore, the  
141 mean serum concentrations of GADA were significantly elevated in patients with  
142 T1DM. Notably, this autoimmune marker showed the greatest increase in the T1DM  
143 group with concurrent bacterial infections. Conversely, the concentration of C-  
144 peptide in both diabetic groups was markedly lower than the average in the healthy  
145 group, strongly reflecting the significantly reduced pancreatic  $\beta$ -cell function  
146 characteristic of type 1 diabetes.

#### 147 *Frequency of total CD19+ B cells in peripheral blood*

148 Flow cytometric analysis revealed a highly significant decrease in the  
149 percentage of total CD19+B cells in both T1DM patient groups compared to the  
150 healthy control group ( $P \leq 0.001$ ), as shown in Figure 2. Notably, this reduction was  
151 more pronounced in the diabetic group with concurrent bacterial infections ( $3.07 \pm$   
152  $0.26\%$ ) compared to the diabetic group without UTIs ( $3.42 \pm 0.46\%$ ). These findings  
153 indicate that concurrent bacterial infections may further exacerbate the depletion of  
154 the overall B cell population in diabetic patients.

#### 155 *Regulatory B cell frequency within B lymphocytes*

156 According to the data shown in Figure 3, a significant decrease in the  
157 proportion of Breg cells was observed in the T1DM group with UTIs ( $P \leq 0.05$ )  
158 compared to the healthy control group. In contrast, while the T1DM group without  
159 UTIs displayed a slight tendency toward a higher mean proportion of Breg cells

160 compared to the other groups, these differences were not statistically significant ( $P$   
161  $> 0.05$ ).

### 162 *Frequency of memory Bregs*

163 As illustrated in Figure 4, flow cytometric analysis revealed a highly  
164 significant decrease in the frequency of memory Breg cells among both groups of  
165 T1DM patients when compared to the healthy control group ( $47.07 \pm 2.78\%$ ). While  
166 the mean frequency dropped significantly to  $26.97 \pm 2.59\%$  in T1DM patients  
167 without UTIs, an even more profound and statistically significant reduction was  
168 observed in T1DM patients with concurrent UTIs, reaching  $21.98 \pm 2.36\%$ . This  
169 significant difference between the two diabetic subgroups indicates that concurrent  
170 bacterial infection further exacerbates the depletion of memory Bregs in T1DM  
171 patients.

## 172 **4 Discussion**

173 Elevated FBG ( $\geq 126$  mg/dL) and HbA<sub>1c</sub> ( $\geq 6.4\%$ ) values, coupled with  
174 diminished C-peptide concentrations, confirm the severe impairment of pancreatic  
175  $\beta$ -cell function and the absolute absence of endogenous insulin secretion typical of  
176 T1DM [45, 5]. The profound multiplication of GADA a primary indicator of  
177 autoreactive B cell activation and  $\beta$ -cell apoptosis was exceptionally pronounced  
178 among diabetic patients experiencing concurrent UTIs [1]. Such an exacerbation  
179 implies that infection-induced inflammatory stress serves as a critical environmental  
180 trigger, intensifying the autoimmune cascade and autoantibody production through  
181 potential pathways like molecular mimicry or bystander activation [16].  
182 Furthermore, focusing on young male cohorts (under 15 years of age) captures the  
183 early, highly active phases of the disease. This specific developmental window is  
184 optimal for tracking critical immunological shifts, especially the progressive  
185 breakdown of immune tolerance mediated by B regs against pancreatic islet antigens

186 [7]. Historically, B cells have been recognized primarily for their roles in antigen  
187 presentation to T lymphocytes and the generation of specific autoantibodies.  
188 Consequently, defects in B cell development and function are highly implicated in  
189 driving autoimmune disorders [4]. Studies by Kendall *et al.* indicated that, during  
190 the initiation of islet inflammation in T1DM mouse models, B cells are among the  
191 first to infiltrate the pancreatic islets. Within these tissues, they organize with T cells  
192 into germinal center-like lymphoid structures, crucially contributing to the selection  
193 and expansion of self-reactive B cells [17]. However, growing evidence highlights  
194 an opposing, protective role mediated by IL-10-producing regulatory B cells  
195 (B10/Breg cells). Breg cells play a crucial role in maintaining immune homeostasis  
196 by secreting IL-10, which dampens inflammation and limits excessive autoimmune  
197 responses against pancreatic  $\beta$ -cells [46]. Mechanistically, these cells downregulate  
198 major histocompatibility complex class II and co-stimulatory molecules, thereby  
199 restricting auto-antigen presentation. Crucially, unlike effector B cells, B10 cells  
200 actively promote the expansion of CD4+CD25+Tregs, while simultaneously  
201 repressing the differentiation of pro-inflammatory Th1 and Th17 cells, and  
202 inhibiting the antigen-presenting capacity of dendritic cells [9, 8]. The marked  
203 decrease in the frequencies of total B lymphocytes, overall Bregs, and specifically  
204 memory Bregs in children with T1DM compared to the healthy control group  
205 actively contributes to the severe impairment of peripheral immune tolerance and  
206 the unabated progression of the disease [6,39]. Elevated levels of memory B cells  
207 are typically associated with the immune system's ability to prevent localized  
208 inflammation from escalating into severe, systemic autoimmune tissue damage [54].  
209 These memory Bregs perform rapid regulatory functions upon antigen re-exposure;  
210 they are activated in the early stages of inflammation to stabilize the immune  
211 response before Tregs fully emerge [25]. Previous studies in humans and murine  
212 models demonstrate a "depletion and return" pattern, where these regulatory cells  
213 are exhausted as inflammatory responses escalate [34]. Since memory B cells are  
214 highly effective at regulating both innate and adaptive immunity including

215 controlling the activity of helper T cells, macrophages, and natural killer cells their  
216 depletion in T1DM leaves the pancreatic tissue highly vulnerable to sustained  
217 immunological damage [12,28,36]. Furthermore, our data revealed an even more  
218 profound suppression in the frequency of these B lymphocyte subsets in T1DM  
219 patients suffering from concurrent UTIs compared to both non-infected diabetic  
220 patients and healthy controls. This reflects the dual of B cells in the diabetic milieu:  
221 they must manage both the chronic autoimmune response and the acute innate  
222 immune response against bacterial virulence factors [30,48]. The data strongly  
223 suggest that certain bacterial pathogens may induce apoptosis in B cells or skew the  
224 immune compartment towards a purely pro-inflammatory phenotype, thereby  
225 stripping away the protective Breg layer and increasing susceptibility to severe  
226 immune dysregulation [14,26]. Consequently, the severe numerical and functional  
227 reduction of Bregs resulting from concurrent bacterial infections significantly  
228 exacerbates the loss of immune homeostasis, accelerating  $\beta$ -cell destruction in  
229 T1DM [53,50]. This provides a strong rationale for future studies exploring memory  
230 Bregs as potential targets for advanced immunotherapeutic interventions.

## 231 **5 Conclusion**

232 In conclusion, the frequencies of total B cells, Breg cells, and specifically  
233 memory Bregs are significantly diminished in the peripheral blood of children with  
234 T1DM compared to healthy controls. Crucially, this depletion is significantly  
235 exacerbated in T1DM patients suffering from concurrent bacterial infections (UTIs).  
236 These findings indicate that infection-induced inflammatory stress further exhausts  
237 the Breg pool. Ultimately, this profound numerical decline underscores the role of  
238 Breg cells in the loss of peripheral immune tolerance in pediatric T1DM,  
239 highlighting these cells as highly promising targets for future immunotherapeutic  
240 interventions aimed at restoring immune homeostasis.

## 241 **Recommendations:**

242 Based on the findings of this study, we propose the following  
243 recommendations:

244 1. Implement longitudinal monitoring of Bregs frequencies and  
245 functions in pediatric T1DM patients particularly during episodes of bacterial  
246 infection to evaluate their prognostic value in disease progression.

247 2. Develop targeted immunotherapeutic strategies aimed at  
248 restoring or expanding Breg cell populations, with the ultimate goal of  
249 preserving residual pancreatic  $\beta$ -cell function and re-establishing immune  
250 tolerance.

251 3. Expand future cohort studies to include larger sample sizes and  
252 diverse age groups, allowing for a more comprehensive understanding of how  
253 concurrent bacterial infections drive systemic immune dysregulation across  
254 different stages of T1DM pathogenesis.

#### 255 **Additional information**

256 **Declaration of competing interest.** The authors declare that they have no  
257 known competing financial interests or personal relationships that could have ap-  
258 peared to influence the work reported in this paper.

259 **Acknowledgments.** The researchers extend their sincere thanks and  
260 appreciation to the medical and nursing staff at Imam Hussein Hospital for their  
261 invaluable cooperation in collecting blood samples. They also thank the staff at Zain  
262 Al-Abidin Hospital for providing the necessary facilities for operating the flow  
263 cytometry device. Furthermore, they express their deep gratitude to all patients and  
264 their families for their voluntary participation in this study.

**ТАБЛИЦЫ**

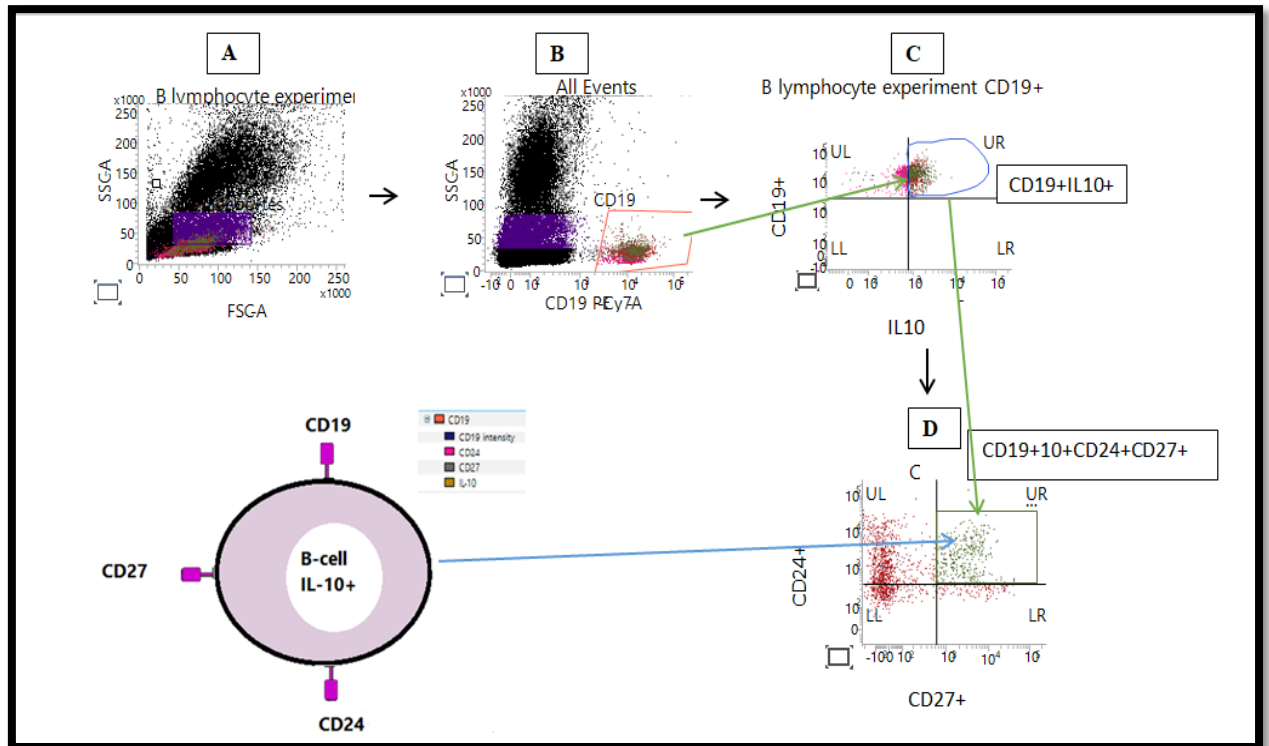
**Table 1.** Clinical and Demographic Characteristics of the Study Groups.

<b>Parameter</b>	<b>Healthy individual (n = 30)</b>	<b>T1DM without UTIs (n = 30)</b>	<b>T1DM with UTIs (n = 30)</b>	<b>P-value</b>	<b>Sig.</b>
<b>Age (years)</b>	10.3 ± 0.4	10.6 ± 0.3	9.9 ± 0.5	> 0.05	Non-Sig.
<b>FBG (mg/dL)</b>	92.4 ± 3.1	185.6 ± 12.3	250.00 ± 19.76	≤ 0.001	Sig.
<b>HbA<sub>1c</sub> (%)</b>	5.2 ± 0.1	8.4 ± 0.3	10.43 ± 0.40	≤ 0.001	Sig.
<b>GADA (U/mL)</b>	4.1 ± 0.8	215.3 ± 42.1	364.5 ± 123.7	≤ 0.001	Sig.
<b>C-peptide (ng/mL)</b>	1.85 ± 0.12	0.210 ± 0.05	0.176 ± 0.07	≤ 0.001	Sig.

**Note.** Data are presented as mean  $\pm$  standard error of the mean (SEM). T1DM = Type 1 Diabetes Mellitus; UTIs = Urinary Tract Infections; FBG = Fasting Blood Glucose HbA<sub>1c</sub> = Glycated Hemoglobin; GADA = Glutamic Acid Decarboxylase Autoantibodies. *P*-value = Probability value; Sig = **Statistically significant**.

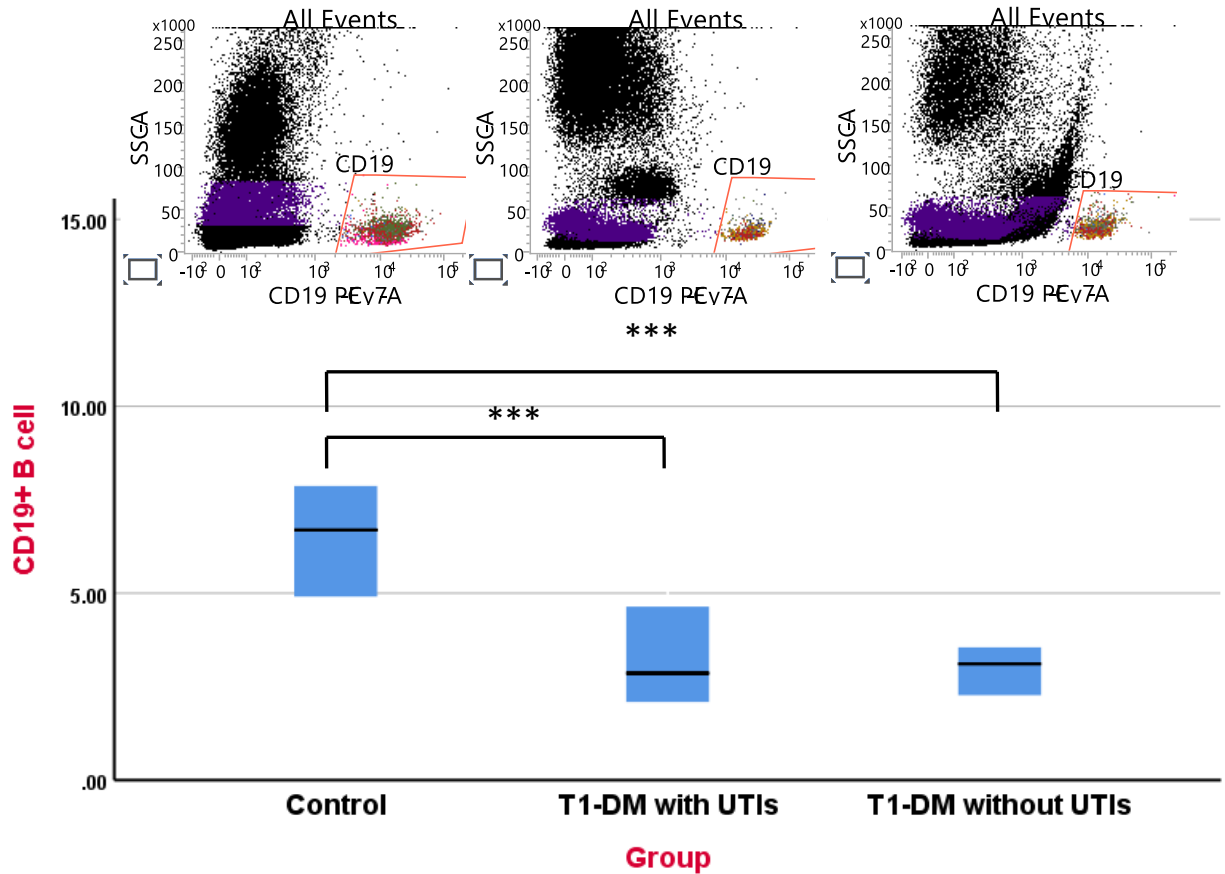
## РИСУНКИ

**Figure 1.** Flow Cytometry Gating Strategy for Identifying CD19<sup>+</sup> IL-10<sup>+</sup> CD24<sup>hi</sup> CD27<sup>+</sup> Memory Bregs.



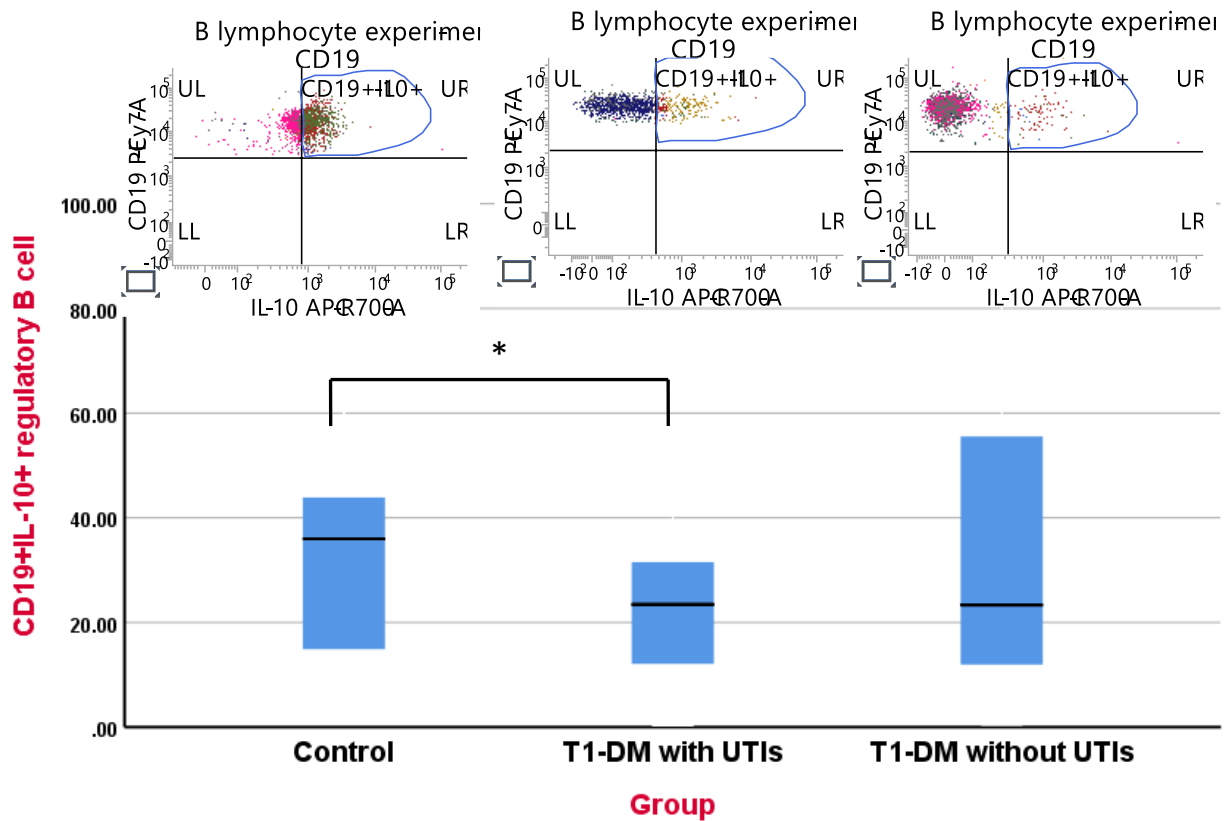
**Note:** (A) Total lymphocytes were initially gated based on their forward scatter (FSC) and side scatter (SSC) characteristics within the single-cell population extracted from the isolated peripheral blood mononuclear cells (PBMCs). (B) Total B cells were then identified by gating on the CD19<sup>+</sup> population within the previously defined lymphocyte gate. (C) Intracellularly stained IL-10-producing B cells (CD19<sup>+</sup> IL-10<sup>+</sup>) were subsequently identified. (D) Finally, the specific percentage of memory Bregs expressing the complete phenotype (CD19<sup>+</sup> IL-10<sup>+</sup> CD24<sup>hi</sup> CD27<sup>+</sup>) was calculated.

**Figure 2.** Comparison of the frequency of total CD19+ B cells among T1DM patients with urinary tract infections (T1DM with UTIs), T1DM patients without UTIs, and a healthy control group.



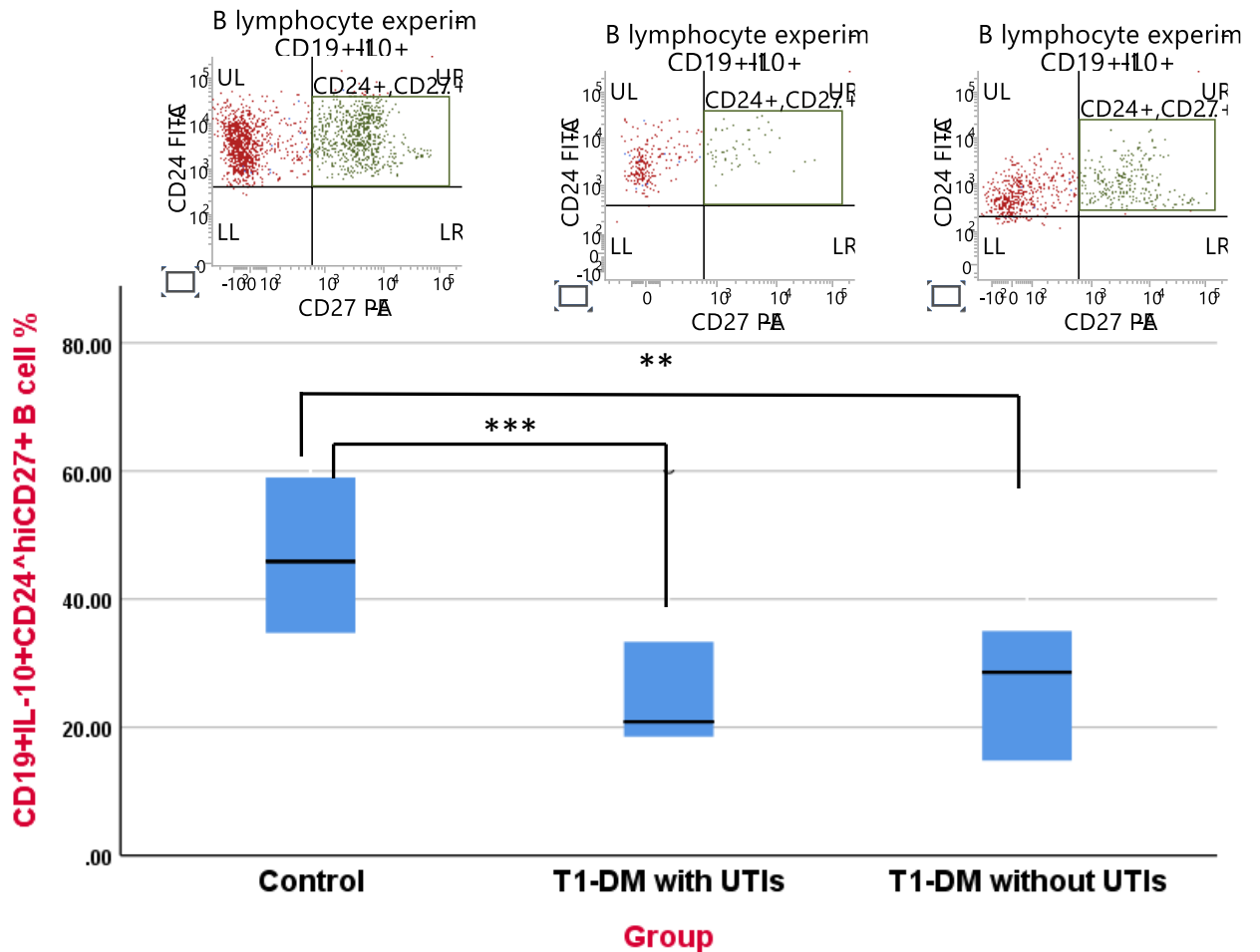
**Note.** Statistical significance is indicated by asterisks (\*), where \*\*\* indicates  $P \leq 0.001$ .

**Figure 3.** Comparison of CD19+IL-10+ regulatory B cell frequencies among T1DM patients with urinary tract infections, T1DM patients without urinary tract infections, and a healthy control group.



**Note:** Statistical significance is indicated by asterisks (\*) as follows: \*  $P \leq 0.05$ .

**Figure 4.** Comparison of the frequency of CD19+ IL-10+ CD24<sup>hi</sup> CD27+ memory Bregs among the healthy control group, T1DM patients with UTIs, and T1DM patients without UTIs.



**Note:** Statistical significance between groups is indicated by asterisks (\*), corresponding to the following probability levels: \*\*  $P \leq 0.01$  and \*\*\*  $P \leq 0.001$ .

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Russian Journal of Infection and Immunity

ISSN 2220-7619 (Print)

ISSN 2313-7398 (Online)

**B-CELLS IN T1DM AND UTI RESPONSE**

**В-КЛЕТКИ ПРИ САХАРНОМ ДИАБЕТЕ 1 ТИПА И ИНФЕКЦИИ МОЧЕВЫВОДЯЩИХ ПУТЕЙ**

**10.15789/2220-7619-DPP-18160**

Учреждение: Кафедра биологии, факультет естественных наук, Университет  
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DETERMINING PARTICIPATION PERIPHERAL BLOOD B-CELLS, B-REG CELLS, AND MEMORY B-REG CELLS IN DIABETES MELLITUS TYPE I DISEASE AND THE EFFECT OF THE URINARY TRACT INFECTION RESPONSE

ОПРЕДЕЛЕНИЕ РОЛИ ПЕРИФЕРИЧЕСКИХ В-КЛЕТОК КРОВИ, В-РЕГУЛЯТОРНЫХ КЛЕТОК И В-РЕГУЛЯТОРНЫХ КЛЕТОК ПАМЯТИ ПРИ САХАРНОМ ДИАБЕТЕ 1 ТИПА И ВЛИЯНИЕ ОТВЕТА НА ИНФЕКЦИЮ МОЧЕВЫВОДЯЩИХ ПУТЕЙ

**Сокращенное название статьи для верхнего колонтитула:**

B-CELLS IN T1DM AND UTI RESPONSE

В-КЛЕТКИ ПРИ САХАРНОМ ДИАБЕТЕ 1 ТИПА И ИНФЕКЦИИ МОЧЕВЫВОДЯЩИХ ПУТЕЙ

**Keywords:** B-cells, regulatory B-cells, memory B-cells, Type 1 Diabetes mellitus, urinary tract infection, immune response.

**Ключевые слова:** В-клетки, регуляторные В-клетки, В-клетки памяти, сахарный диабет 1 типа, инфекция мочевыводящих путей, иммунный ответ.

Оригинальные статьи.

Количество страниц текста – 10,

количество таблиц – 1,

количество рисунков – 4.

01.03.2026

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