Chapter

Splenectomy in Gastric Cancer: Influence of B Lymphocytes

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Abstract

The spleen is the largest peripheral organ of the immune system. The standard volume of lymphodissection in stomach cancer during gastrectomy or proximal resection is D2, which implies splenectomy. Immunity disorders in patients after splenectomy primarily affect the B cell immune response. Peripheral blood B-lymphocytes subpopulations have been studied in patients with gastric cancer. Group 1 - patients with gastrectomy, D2 lymphodissection, group 2 - patients with gastrectomy, D2-lymphodissection, splenectomy. Evaluation of the expression of antigens (CD20, CD21, CD23, CD38, HLA-DR, CD71, CD10, CD95, CD25, CD5, CD56, κ- and λ-light was performed in the gate of CD19+ cells. Among peripheral blood lymphocytes the presence of CD19+CD5+ B cells (B1a cells), some of which express the activation antigens CD38 and CD23 is found; a small part of CD5+ B cells is CD25+CD38−. The number of CD23+ cells ranged from 25 to 40% in different patients. A significant number of B cells with a low level of CD21+ expression were detected. In group 2 after surgery, the percentage of cells with CD5+ expression significantly increased, the relative amount of CD19+ lymphocytes, CD19+CD21+ B cells decreased. Given data on B1 and BMZ populations, this can lead to a weakening of both general and antitumor immunity.

Keywords: B lymphocytes, B1 cell, cells of the marginal zone of the spleen, thymus-independent antigens, humoral immunity, stomach cancer, splenectomy, splenomegaly, D2 lymphodissection, IgM

1. Introduction

The spleen is one of the most important peripheral organs of the immune system. The content of lymphocytes in the white pulp of the spleen reaches 85% of the total number of cells. This amount is almost 25% of all body's lymphocytes, almost half of them being B cells. Thus, it is the spleen, along with the lymph nodes, which is the organ providing humoral immunity. In the spleen, a red pulp of 70–80% of the body weight is distinguished and white pulp, which accounts for 6–20% of the mass. The red pulp of the spleen is represented by venous sinuses and pulpal strands. In the red pulp, destruction of red blood cells

and their absorption by macrophages occur. Lymphocytes predominate in the white pulp of the spleen. They accumulate around the arterioles in the form of so-called periarteriolar clutches. B cell follicles are located closer to the edge of the clutch [1–3].

Complex processes of activation of B cells occur in the spleen. Immature B cells come from the bone marrow to the spleen and lymph nodes, where their further maturation, antigen presentation, proliferation, and differentiation occur. Molecules of IgD, CD21, and CD22 appear on their surface.

The process of activation of B cells can be carried out in response to thymus-dependent antigens or without the involvement of a T lymphocyte [4]. T-independent antigens are generally polyvalent lipopolysaccharides, polysaccharides, or proteins. T-independent antigens are divided into type I and type II antigens, which differently ensure the full development of B cells in cells that synthesize antibodies [5–8].

Immature B cells react to T cell-independent antigens of type 1, which elicit a rapid antibody response. Most of mature B cells are within the lymphoid follicles of the spleen and lymph nodes, where they collide and react to T-dependent antigens associated with follicular dendritic cells and proliferate and either differentiate into plasma cells [1].

B cells, specific for autologous antigens, do not enter the follicles; they linger in the outer zone of the periarteriolar lymphoid clutches and die [9]. During the immune response to various antigens, the B lymphocyte-specific immunoglobulin receptor is bound after which the movement of all B cells in the outer zone of the periarteriolar lymphoid clutches has significantly slowed down. Activated B lymphocytes die in the event that there is no interaction with the T cells necessary for the realization of an immune response to thymus-dependent antigens. In the presence of cooperation with T cells, naive B cells enter mainly into follicles, where they undergo differentiation in the germinal centers during primary immune responses.

In secondary immune responses of memory B cells to thymus-dependent antigens, pronounced B cell proliferation and differentiation into plasma cells within the outer zone of periarteriolar lymphoid clutches are observed; follicular B cell proliferation is somewhat weaker than with primary responses [10, 11].

Also in the spleen, there is a special population of cells that delimits white pulp from the red pulp. This area is called the marginal or marginal zone, where both T and B cells are located with a relative predominance of the latter [12]. The population of the B cells of the marginal zone is not homogeneous: it includes naive B cells, as well as B cells of immunological memory, generated both during T-dependent and T-independent antibody responses of the first type [13].

The spleen is the main place for the synthesis of IgM [14–16]. IgM is a polymer in which multiple immunoglobulins are linked together by covalent bonds known as disulfide bonds. IgM-class antibodies are the earliest in immunogenesis and constitute about 6% of all immunoglobulins. The time of their half-life is 5–6.5 days. They are produced by activated B cells at a primary immune response in peripheral lymphoid organs, which also includes lymph nodes and lymphoid formations of mucous membranes [17].

At the same time, the spleen cells are capable of producing various cytokines. In the experiment, it was shown that splenocytes produce interleukin-2, interferon-gamma, and interleukin-7 during antigen stimulation, which in turn stimulate the proliferation of B cells and the production of immunoglobulins [18].

Surgical treatment of patients with gastric cancer involves the implementation of lymphodissection. The standard volume of lymphodissection for stomach cancer

during gastrectomy or proximal resection is D2, which implies a splenectomy that is performed to completely remove the lymph nodes of the spleen gates. However, the splenectomy leads to an increase in the frequency of postoperative complications and mortality, as confirmed by European randomized trials [19–23].

The spleen is the largest peripheral organ of the immune system. Immunity disorders can be more pronounced and prolonged after splenectomy. Clinical observations indicate that the improvement of health and clinical and laboratory parameters after splenectomy is in some cases replaced by the development state of immunodeficiency. Currently, this is confirmed experimentally and is referred to in the foreign literature as overwhelming postsplenectomy infections (OPSI) [24–26]. An analogue of the name "overwhelming postsplenectomy infections" is the term "postsplenectomy hypersplenism"; the signs of which are decrease in general tone and performance and susceptibility to viral, bacterial, parasitic, and fungal infections [26, 27].

Splenectomy for the purpose of adequate lymphodissection in stomach cancer causes pronounced and long-term dysfunction of various immunity units: the presentation of macrophages by foreign antigens to T and B lymphocytes is disrupted, the subpopulation of B lymphocytes changes, and the levels of all classes of immunoglobulins (IgG, IgA, IgM) decrease [28–30]. However, immunity disorders in patients after splenectomy primarily affect the B cell immune response, including thymus-independent type 2 antigens, which is provided by the population of B1 lymphocytes [31, 32].

B1 lymphocytes are relatively small group of B cells, found in humans and mice, and are considered to be the most phylogenetically oldest branch of antibody-producing cells. The population of B1 cells was first described in 1983 by Lee Herzenberg (Hayakawa et al.) as a CD5+ population that differs from normal B (B2) cells by phenotype, anatomical localization, self-healing ability, and the production of natural antibodies. It includes two subpopulations: B1a and B1b [32, 33]. B1 lymphocytes develop in the fetal liver from progenitor cells.

The predecessors of B1a lymphocytes in ontogenesis appear before other subpopulations and migrate from the embryonic hematopoietic tissues (fetal liver and omentum) to the abdominal and pleural cavities as early as the embryonic period. B1b lymphocytes also originate from fetal precursors, but their pool in adults can be partially replenished by the bone marrow and migrate to the serous cavities during the embryonic period where they exist throughout the life of the organism [34, 35].

Thus, during life, the B1 lymphocyte pool is maintained by the activity of progenitor cells through their very slow proliferation. B1 cells are localized mainly in serous cavities—the abdominal and pleural. Some B1 cells migrate (through the omentum) to the intestinal mucosa and mesenteric lymph nodes (up to 50% of IgA producers in the lymphoid tissue of the intestinal B1cells). In the lymph nodes of the mouse, they are absent.

B1 cells are characterized by an "activated phenotype," which is manifested in the expression on their surface of costimulatory molecules CD80 and CD86. This property provides the ability of B1 lymphocytes to function as antigen-presenting cells. Subpopulations of B1 lymphocytes are similar, but subpopulation of B1b is characterized by the absence of CD5 expression [36].

The antibodies produced by B1 lymphocytes are almost exclusively IgM. The response of B1 cells is predominantly thymus-independent [37]. IgM plays an important role in the induction of apoptosis of tumor cells [38–40]. Approximately half of the serum IgM is secreted by B1 cells. A small number of B1 lymphocytes, mainly cells secreting antibodies, are detected in the spleen, where they account for up to 5% of the number of B cells.

To study the peculiarities of the B cell link in patients with gastric cancer after splenectomy, the subpopulation composition of B lymphocytes was analyzed. The study included two groups of patients who underwent surgery. The first group of patients received treatment in the volume of gastrectomy with spleen-protective D2 lymphodissection. In the second group, patients underwent gastrectomy with D2 lymphodissection and splenectomy. The study of subpopulations of B lymphocytes was carried out at the preoperative stage and 3 months after the surgical treatment.

2. Materials and methods

We studied the subpopulation composition of B lymphocytes of peripheral blood in patients with gastric cancer. The possible influence of splenectomy on the subpopulation composition of B lymphocytes was investigated. The B cell link of peripheral blood lymphocytes in patients with gastric cancer was studied in dynamics (3 months after the operation).

To assess the effect of the splenectomy on the B cell link, the subpopulation composition of B cells of peripheral blood after surgery was assessed both in patients after gastrectomy with standard D2 lymphodissection (splenectomy) (14 people) and in patients after gastrectomy with spleen-protective D2 lymphodissection (12 people).

The reaction was taken into account on flow cytometers (FACScan, Lysys II and FACSCanto II, FACSDiva program). Data processing: WinMDI 2.8 and FCS 3.0 applications. Evaluation of the expression of membrane antigens was carried out in the gate of CD19+ B cells. Cells were stained at the same time with three monoclonal antibodies labeled with different fluorochromes. In 42 peripheral blood samples before the operation and 23 samples after the operation on the hematological analyzer Micros 60, the hemogram was calculated, which allowed to estimate the absolute number of B cells.

The expression of the following antigens is analyzed: CD20, CD21, CD23, CD38, HLA-DR, CD71, CD10, CD95, CD25, CD5, CD56, IgG-λ, and IgG-κ light chain immunoglobulins (**Table 1**). Direct conjugates of monoclonal antibodies with fluorochromes were used: FITC, fluorescein; PE, phycoerythrin; PerCP, peridinin

Antigen	Antibody clone	Antibody isotype	Manufacturer	Fluorochrome
CD19	4G7, HIB19	IgG1-κ	BD Biosciences	PerCP
CD20	2H7	IgG1-κ	BD Biosciences	PE
CD23	LT23	IgG1-κ	Sorbent, Russia	FITC
CD5	L17F12	IgG2a-κ	BD Biosciences	PE
CD21	BL13	IgG1-κ	BD Biosciences	FITC
CD10	HL10a	IgG2a-κ	BD Biosciences	PE
CD71	L01.1	IgG2a-κ	BD Biosciences	FITC
CD95	DX2	IgG1-κ	BD Biosciences	PE
CD25	2A3	IgG1-κ	BD Biosciences	FITC
CD56	NCAM 16.2	IgG2a-κ	BD Biosciences	PE
CD3	SK7	IgG1-κ	BD Biosciences	FITC
IgG-λ/IgG-κ	—	—	BD Biosciences	PE/FITC

Table 1.
Monoclonal antibodies and antigens.

chlorophyll protein; and PE-Cy5, double (tandem) dye-combining phycoerythrin with cyanine 5. The expression of the above antigens was detected by monoclonal antibodies directly labeled with various fluorochromes.

3. The effect of the splenectomy on humoral immunity in patients with gastric cancer

An analysis of the B cell subpopulation was performed in the gate of CD19+ cells. At the first stage of the analysis, the number of CD19+ B cells within the lymphocytes was cytometrically estimated (**Figure 1a**). Further, within the limits of only B cells [gate CD19+, **Figure 1b**], the expression of two antigens associated with B cells was analyzed. In these samples, there is an estimate of CD56 antigens concurrently with CD21 (**Figure 1c**).

The main indices of peripheral blood samples before and after surgery are given in **Table 2**. In general, in the group before the surgical intervention in comparison with the physiological norm, the average indices of both the relative quantity and the absolute content of B cells fit within the limits of the norm.

In 33% of patients with gastric cancer at the preoperative period, a decrease in the relative number of B cells (less than 5%) were detected. Moreover in 38% of patients decrease in the absolute number were revealed. Three months after the operation, in 52% of cases, the relative number of B cells was reduced, in 31% of the absolute content of B cells.

The average relative number of B cells, as well as their mean absolute content in 1 μl of peripheral blood, was slightly elevated 3 months after surgery in comparison with these parameters in preoperative assessment. The average percentage of B lymphocytes after the operation was slightly lower than before surgery. However, these differences were statistically unreliable (T-test when comparing two independent variables). This can be explained by a significant spread of the analyzed indicators both in patients before and after surgery.

3.1 B2 cells

Investigation of subpopulations of peripheral blood B lymphocytes in patients with gastric cancer at the preoperative stage established that all B cells had immunophenotype of naïve mature B2 cells: CD19+ CD20+ HLA-DR+ CD10-CD21low/+. The data of subpopulation composition of B lymphocytes of peripheral blood of gastric cancer patients are given in **Table 3**. B2 cells have a long life, localized mainly

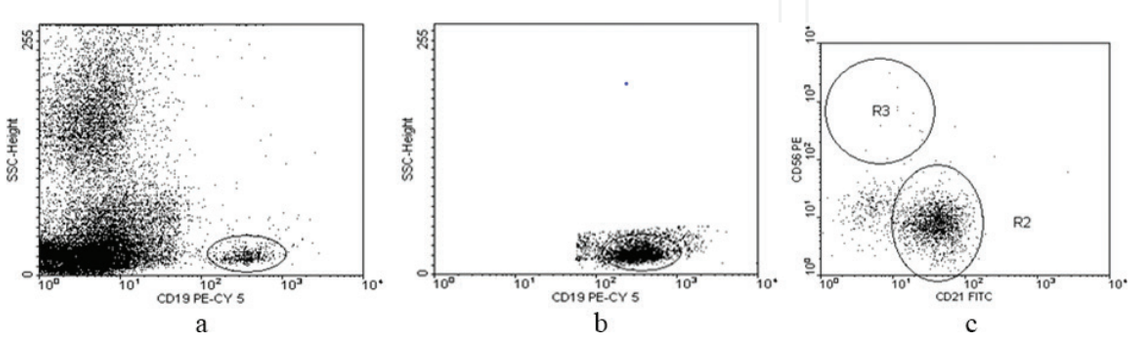


Figure 1.
(a) Estimating the total number of CD19+ B cells within the lymphocytes. The abscissa is the expression of pan-B cell antigen CD19+; along the ordinate axis—the parameter of lateral light scattering of the laser beam, reflecting the working cytometric concept—the granularity of the cells. (b) Cells that fall only in the region of CD19+, i.e., B cells accumulated during sample collection. (c) Evaluation of the expression of two antigens associated with B cells in the CD19+CD56 and CD21.

	Before operation		After the operation	
	Average	Median	Average	Median
Leucocytes (×10^3/μL)	6.3 ± 0.34 n = 43	5.9	6.76 ± 0.49 n = 24	6.4
Lymphocytes (%)	30.1 ± 1.8 n = 43	30.0	36.9 ± 3.06 n = 24	35.5
B lymphocytes (CD19+) %	6.44 ± 0.51 n = 48	5.5	6.0 ± 0.57 n = 28	4.8
B leucocytes (CD19+) %	2.12 ± 0.2 n = 42	2.04	2.5 ± 0.4 n = 22	1.86
B cells (CD19+)/μL	122.5 ± 10.8 n = 42	110.0	156.4 ± 21.5 n = 22	131.0

Table 2.
Parameters of peripheral blood of the general group of patients.

in the spleen, bone marrow, lymph nodes, Peyer's patches, and individual follicles of the lymphoid tissue of the intestine. The histological unit, which is the site of the concentration of B2 cells, is the lymphoid follicle. These cells constitute the vast majority of circulating B lymphocytes. B2 cells undergo selection in the bone marrow and participate in the formation of an adaptive humoral immune response to thymus-dependent antigens.

Some patients noted the presence of transient T2B and T3B cells. They are characterized by pronounced expression of CD23 and CD21. The number of CD23+ cells ranged from 25 to 40% in different patients. In this case, as a rule, CD23+ B cells had a weaker expression of the antigen of mature B cells of CD20. **Figure 2a** shows samples of peripheral blood with a pronounced proportion of CD23+ B cells. In 40% of cases, CD23 coexpression was more than 25%, and in 22.4% of patients, the number of CD23+ B cells exceeded 40% of B cells. Some patients have an insignificant number of CD23+ B cells (6%) (**Figure 2b**).

Transient (immature) B cells were first characterized in mice [41]. Immature B cells migrate from the bone marrow to the spleen. Throughout their migration to the spleen and after spleen entry, they are considered T1 B cells; within the spleen, T1 B cells transition to T2 B cells. Under normal conditions, CD19+CD21lowCD23 B cells (T1-transient) pass positive selection in the course of B cell ontogeny: cells receive a signal through the B cell receptor without the participation of any

	M ± m	Median
CD19+CD5+	14.2 ± 1.91	9.8 n = 46
CD19+CD23+	24.5 ± 2.4	20.5 n = 49
CD19+CD20+	97.2 ± 0.4	97.5 n = 48
CD19+CD71+	9.6 ± 1.1	7.7 n = 48
CD19+CD10+	0.48 ± 0.09	0.2 n = 45
CD19+CD38+	19.8 ± 2.9	11.1 n = 45
CD19+HLA-DR+	98.6 ± 0.3	99.1 n = 48
CD19+CD25+	2.1 ± 0.82	0.5 n = 40
CD19+CD95+	9.6 ± 1.96	7.8 n = 31
CD19+CD21+	80.6 ± 1.43	82.1 n = 43
CD19+CD56+ (IgG-κ+/IgG-λ+)	2.7 ± 2.0	0.15 n = 40, 1.5–2.0 n = 29, 1.0–1.3 n = 12

Table 3.
Subpopulations of B lymphocytes of peripheral blood in patients' stomach cancer before surgery.

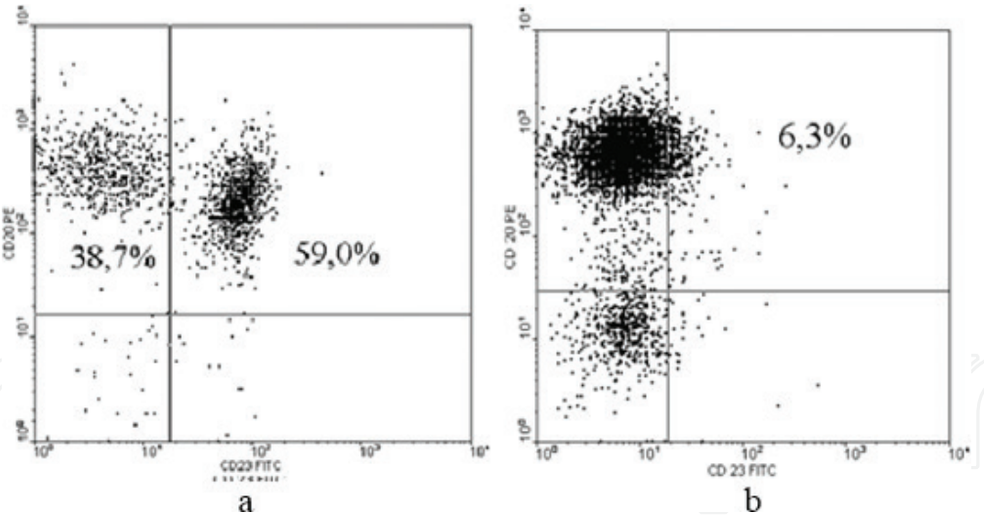


Figure 2.
Expression of CD23 antigen on peripheral B cells in cancer patients'stomach. (a) The population of CD20+CD23+ is the majority (59%) of B cells and represents as a separate discrete population. (b) Insignificant number of CD23+ B cells.

antigens, which ensures their survival. This process can occur both in the bone marrow and in the spleen [42, 43]. In the evaluation of subpopulations of peripheral blood B cells in patients with gastric cancer before surgery treatment, a significant number of B cells with a low level of CD21+ expression were detected [area R2, **Figures 3** and **4**]. This phenotype corresponds to the T1 transient stage of B cell development. Expression of CD21 antigen was characteristic of a larger number of B cells and averaged 82%.

The selection of transient T2B and T3B cells, characterized by the expressed expression of CD23 and CD21 antigens, passes in the peripheral lymphoid organs (the spleen, lymph nodes) high levels of IgM, IgD, and CD23 and lower levels of CD21. The expressed expression of CD23 antigen is intrinsic to the B cells of the embryonic center of the follicle. The follicular B cells that make up the majority and B cells of the marginal zone are lining outside the marginal sinus and bordering the red pulp. Follicular B cells also express high levels of IgM, IgD, and CD23 and lower levels of CD21. These cells are absent in CD1 or CD5, which distinguishes them from B1 cells and B cells of the marginal zone.

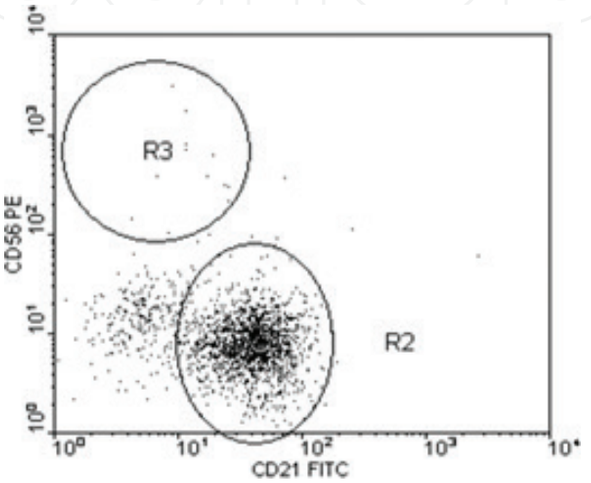


Figure 3.
The population of CD19+CD21low B cells (region R2).

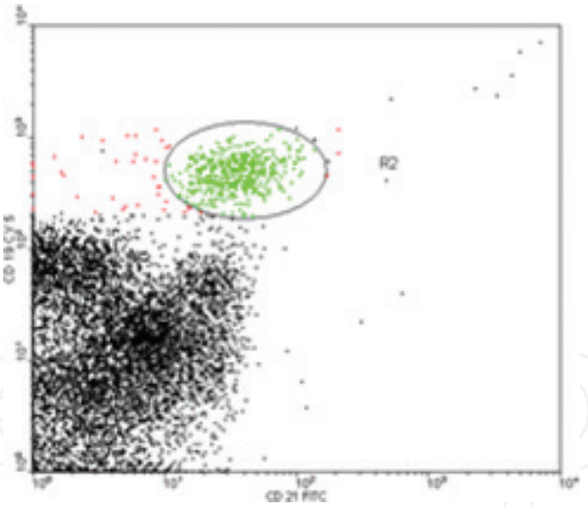


Figure 4.
Expression of CD21 antigen on B cells of blood of patient with stomach cancer. The abscissa axis is the expression of CD21; on the ordinate axis—CD19 expression, in the figure all cells of the sample, including granulocytes. Area R2, CD19+CD21+ cells, this region is located above the granulocytes, which indicates a weak expression of CD21 B cells.

Thus, in patients with gastric cancer, a violation of the immunological repertoire of B2 cells was established: the presence of a pronounced proportion of CD21+ B cells with weak expression, a significant number of CD23+ cells, and cases of clonal B cells. In most of the studied samples, B cells were polyclonal with a predominance of Ig-κ (**Figure 5**). When the thymus-dependent pathway of the immune response is realized, these patients will have a disruption in the synthesis of antibodies.

It is known that a B cell can be activated without the involvement of a T lymphocyte, which leads to the full development of B lymphocytes to antibody-producing forms. It is a characteristic that in this case the immunoglobulin M is synthesized predominantly [5–7]. The process of activation of B cells without T lymphocyte is provided by thymus-independent antigens of type I and II [4]. They are lipopolysaccharides, polysaccharides, and proteins.

Antibodies of IgM class are the earliest in immunogenesis and make up about 6% of all immunoglobulins; their half-life is 5–6.5 days. Approximately half of the

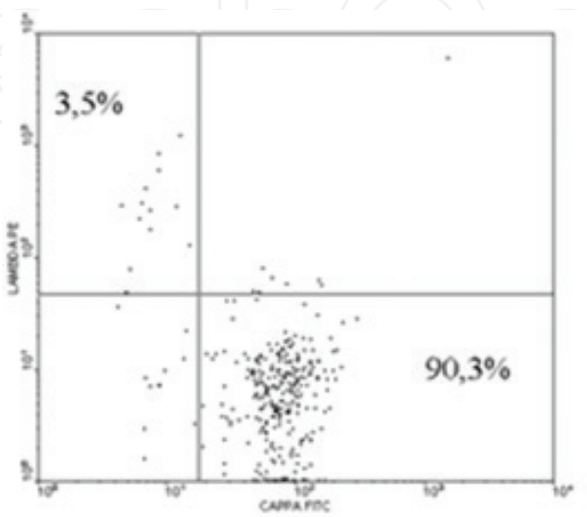


Figure 5.
Polyclonality of B cells with predominance of Ig-κ.

serum IgM is secreted by a pool of B1 lymphocytes. Many of the antibodies are polyspecific, i.e., are able to interact with several antigens, including autologous ones. These antibodies have a low affinity for antigens, including autoantigens, and are not capable of causing damage to tissues. B1 cells are constantly circulating between the spleen and the abdominal cavity but do not enter the follicles, since they do not express the chemokine receptor. Therefore, the processes of "improving" the humoral immune response in the form of switching isotypes and increasing the affinity for antigens do not affect or minimize B1 cells.

3.2 B1 cells

The population of B1 cells is the most phylogenetically ancient branch of antibody producing cells found in humans and mice. B1 lymphocytes develop in the liver of the fetus from progenitor cells. The B1 precursor, which differs from the B-line progenitor that develops primarily in the B2 population, is identified in the murine bone marrow to a lesser degree in the adult bone marrow [34]. B1 cells are considered as key players of the early humoral response against pathogens and considered primary antibody producers in response to T cell-independent type 2 antigens [44]. A specific thymus-independent response is realized by two subpopulations of B1 cells: B1a and B1b.

Both subpopulations of B1 cells are characterized by an "activated phenotype," which is manifested in the expression on their surface of costimulatory molecules CD80 and CD86. This property provides the ability of B1 lymphocytes to function as antigen-presenting cells. B1b cells are phenotypically similar to B1a cells, but they are characterized by the absence of CD5 expression. B1b lymphocytes realize an adaptive immune response, and B1a lymphocytes produce natural antibodies that are specific to microbial agents and opsonize pathogen-mediated innate immunity [45–47].

Such a B1a population of lymphocytes has the phenotype CD19+CD21lowCD23-CD5+IgM++. A number of experimental studies have revealed that immunity disorders after splenectomy primarily affect the B cell immune response, including thymus-independent (TN) type 2 antigens, which is provided by the population of B1a lymphocytes [31, 32].

Among B lymphocytes of peripheral blood of patients with gastric cancer, expressed subpopulation of CD5+ cells was noted. The number of CD19+CD5+ B cells averaged 17.7%. In 23% of patients, CD19+CD5+ lymphocytes were more than 20%, and in three patients, more than 40% of CD19+CD5+ lymphocytes were detected. Normally, the population of CD19+CD5+ B cells is no more than 10% of the total number of peripheral blood B lymphocytes. Among the B cells of the group with a higher CD19+CD5+ B lymphocyte count (more than 15%), the percentage of cells expressing CD25+ and CD21+ antigens was significantly higher; differences in the number of CD38+ B cells were found to be significant, but the range of values for one group of samples with respect to this antigen was higher. The data are presented in **Table 4**.

The presence of CD38 and CD25 antigens on circulating peripheral B cells indicates their activation. It is possible in coexpression of CD23 antigen, which was observed in a group of samples containing more than 25% of B1a lymphocytes.

A high probability of the presence of the CD19+CD23+CD38+CD5+ population (the presence of a reliable correlation relationship for B cells with the expression of CD38 and CD23 antigens, $R = 0.885$, $p = 0.008$) was established. There was also a high probability of the presence of the CD19+CD5+CD25+ population (significant

Population	Group 1	Group 2	Group 1/group 2	*t*-Test	Relevance
CD21	85.3 ± 1.6	79.1 ± 1.9	13/27	2.436	0.02
CD25	3.9 ± 2.0	0.7 ± 0.1	11/26	2.435	0.02
CD38	27.7 ± 6.5	16.1 ± 2.9	16/38	1.858	0.07

Table 4.
Significant populations of B cells in groups of patients with stomach cancer with (CD5+) lymphocytes (group 1 ≥ 15%) and their normal content (group 2 < 15%).

correlation for CD1+CD5+ and CD19+CD25+ B cells, R = 0.879, p = 0.05). However, in most samples (65%), the number of CD19+CD38+ B cells was insignificant—less than 15.0% (**Figure 3b**).

It is interesting to note that the expression levels of the CD5 antigen on B cells proved to be significantly weaker [region R3, (**Figure 5d**)] as compared to the expression of CD5 antigen on peripheral CD3+ T cells.

Thus, in 17.7% of patients with gastric cancer in the peripheral blood, there is a pronounced proportion of a special subpopulation that provides a specific response to thymus-independent type II antigens, which is accompanied primarily by the synthesis of immunoglobulin M: B1a lymphocytes, some of which express the activation antigens CD38 and CD23. It is known that the precursors of these cells migrate early from the hematopoietic tissue to their anatomical niche, into the abdominal and pleural cavities, where autonomy from the central organs of the immune system is maintained by the number of its population. Some B1 cells migrate (through the omentum) to the intestinal mucosa and mesenteric lymph nodes (up to 50% of IgA producers in the lymphoid tissue of the intestinal B1 cells).

3.3 Cells of the marginal zone

Coexpression of CD38 and CD25 can be observed within the B cell population of the marginal zone of the spleen. B cells of the marginal zone (BMZ) originate from a pool of recycled B lymphocytes that have returned to the marginal zone of the spleen. Phenotypically, these cells are more similar to B2 cells than to B1 cells. They come from the same bone marrow precursor cells. Separation of the line of BMZ cells from the general line of B2 cells occurs in the transitional stage of transient cells (T3), when future BMZ cells weaken the expression of non-IgM (like B2 cells) and IgD and lose the CD23 molecule.

These cells have specific morphological features: IgM molecules are expressed on their membrane, but there are no IgD molecules [48]. IgM is expressed more strongly than on B2 cells. For BMZ, high expression of CD21 is characteristic, which allows them to successfully bind T cell-independent type 2 antigens, presented in particular on encapsulated bacteria [49]. The molecules of CD69, CD25, and CD38 in a small amount of CD23 are expressed on BMZ. B lymphocytes with this phenotype are also found in other lymphoid tissues, but only the marginal zone of the spleen accumulates the largest number of these cells in the body. At the BMZ lymphocytes, the chemokine receptor is not expressed, which allows the cells to migrate to the follicles. The cells of the marginal zone are located in the primary blood sine network of the spleen, which allows them to interact with antigens carried by the blood [38, 51]. Information on antigens of BMZ is obtained by "shuttle" migration to lymphoid follicles and back. When answering antigens, the BMZ cells differentiate into short-lived antibody-forming cells. Due to the strong expression of MHC-11 molecules and costimulatory

molecules, BMZ cells have pronounced an ability to interact with T-helper cells. The period of their life is comparable with the life of an organism.

In the spleen, there are complex processes of differentiation, selection of B lymphocytes, and replenishment of the pool of recirculating B lymphocytes and the pool of BMZ and B1 cells. To evaluate the features of the B cell link of immunity in patients with gastric cancer after gastrectomy with splenectomy, an investigation of subpopulations of B lymphocytes in the dynamics before and after the operation was carried out. Immunophenotypic profile of B cells was studied in 14 patients with gastric cancer with spleen-protective D2 lymphodissection and gastrectomy and in 16 patients with gastric cancer after gastrectomy and D2 lymphodissection with splenectomy.

In the group of patients with spleen-protective D2 lymphodissection, a significant correlation between the relative values (the proportion of lymphocytes and leukocytes) and the absolute (cells in 1 μl of blood) in the number of CD19+ B cells was established in a pairwise comparison (T-test for paired variables) ($p = 0.015$, $p = 0.04$, and $p = 0.05$, respectively). The number of cells in the CD19+CD21+ population ($p = 0.034$) also significantly correlated before and after the operation.

In the group of patients who underwent gastrectomy with splenectomy, a significant correlation between the relative number of B lymphocytes ($p = 0.018$) and CD5+ B lymphocytes ($p = 0.012$) and the number of CD19+CD38+ cells was found in a pairwise comparison of mean values before and after surgery ($p = 0.035$).

Three months after surgery in comparison with preoperative parameters in patients with gastric cancer after splenectomy, the percentage of cells with CD5+ expression significantly increased ($t = -6.015$ sig <0.0001, $p = 0.013$), and the relative amount of CD19+ lymphocytes and CD19+CD21+ B cells was decreased (before surgery, their number was 83.6%, and after, 73.9%, $p = 0.08$). The largest number of CD19+CD21+ B cells in the body is accumulated in the marginal zone of the spleen. When comparing the two groups after surgery, a high percentage of CD19+CD5+ B cells was detected (21.7 vs. 14.5%) in the group of patients with splenectomy ($p = 0.049$), which are precursors of functionally more advanced and clonally more diverse true B cells.

4. Discussion

In the spleen, there are complex processes of differentiation, selection of B lymphocytes, and replenishment of the pool of recirculating B lymphocytes and the pool of BMZ and B1 cells.

Coexpression of CD38 and CD25 can be observed within the B cell population of the marginal zone of the spleen. B cells of the marginal zone (BMZ) originate from a pool of recycled B lymphocytes that have returned to the marginal zone of the spleen. Phenotypically, these cells are more similar to B2 cells than to B1 cells. They come from the same bone marrow precursor cells.

These cells have specific morphological features: IgM molecules are expressed on their membrane, but there are no IgD molecules [49]. IgM is expressed more strongly than on B2 cells. For BMZ, high expression of CD21 is characteristic, which allows them to successfully bind TH2 antigens (T cell-independent type 2 antigens), presented in particular on encapsulated bacteria [50, 51]. The molecules of CD69, CD25, and CD38 in a small amount of CD23 are expressed on BMZ. B lymphocytes with this phenotype are also found in other lymphoid tissues, but only the marginal zone of the spleen accumulates the largest number of these cells in the body.

Information on antigens of BMZ is obtained by "shuttle" migration to lymphoid follicles and back. When responding to antigens, the BMZ cells differentiate into short-lived antibody-forming cells. Due to the strong expression of MHC-11 molecules and costimulatory molecules, BMZ cells have a pronounced ability to interact with T-helper cells.

B1 lymphocytes are small group of B cells, found in humans and mice. CD5+ population differs from B2 cells by their phenotype, anatomical localization, self-healing ability, and the production of natural antibodies, includes two subpopulations: B1a and B1b [32, 33]. B1 lymphocytes develop in the fetal liver from progenitor cells.

The predecessors of B1a lymphocytes in ontogenesis appear before other subpopulations and migrate from the embryonic hematopoietic tissues (fetal liver and omentum) to the abdominal and pleural cavities as early as the embryonic period. B1b lymphocytes also originate from fetal precursors, but their pool in adults can be partially replenished by the bone marrow and migrate to the serous cavities during the embryonic period where they exist throughout the life of the organism [34, 35]. Thus, during life, the B1 lymphocyte pool is maintained by the activity of progenitor cells through their very slow proliferation. B1 cells are characterized by an "activated phenotype" which is manifested in the expression on their surface of costimulatory molecules CD80 and CD86. This property provides the ability of B1 lymphocytes to function as antigen-presenting cells. Subpopulations of B1 lymphocytes are similar, but subpopulation of B1b is characterized by the absence of CD5 expression [36].

The data obtained show a disruption in the composition of B cell subpopulations. Most peripheral blood B cells showed weak levels of CD21 (low) antigen expression, marked presence of a pronounced amount of CD2+ B cells, and cases of clonal B cells. This probably reduces the function of antibody formation in the case of the realization of the main path of development of antibody producers in response to thymus-dependent antigens.

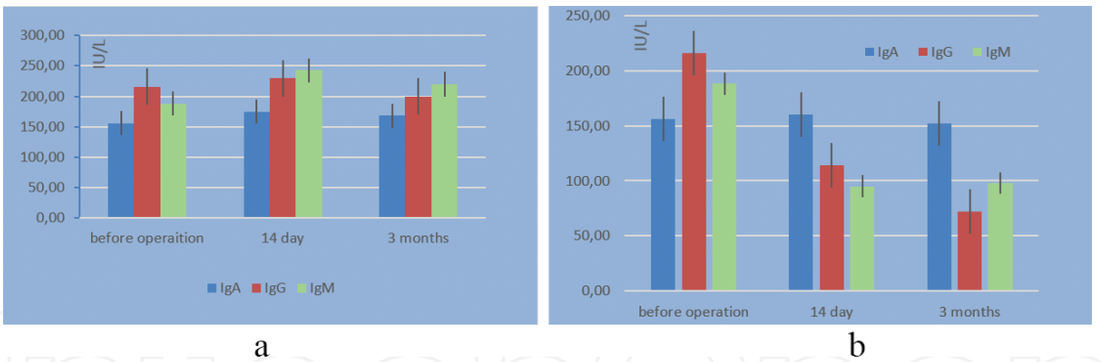
The antibodies produced by B1 lymphocytes are almost exclusively IgM. The response of B1 cells is predominantly thymus-independent [37]. IgM plays an important role in the induction of apoptosis of tumor cells [38–40]. Approximately half of the serum IgM is secreted by B1 cells. A small number of B1 lymphocytes, mainly cells secreting antibodies, are detected in the spleen, where they account for up to 5% of the number of B cells.

In the works which were devoted to the study of the function of the spleen and performed on animals, it has been shown that after splenectomy in the serum, the IgM level and the phagocytic activity of neutrophilic granulocytes decreased. However, if the spleen is reimplanted, the concentration of IgM is increased [52].

In a clinical study conducted at our research center, the levels of the main IgG, IgM, and IgA immunoglobulin classes were studied in patients with gastric cancer who underwent a standard operation in the volume of gastrectomy and lymphadenectomy with splenectomy [53].

The level of immunoglobulins was determined by the method of radial immunodiffusion in Mancini with the use of test systems produced by the SPC Medical Immunology (Russia). The level of immunoglobulins A, G, and M in serum in patients without splenectomy, from 14 days after the operation, was slightly elevated and decreased to the initial value by 3 months (**Figure 6a**).

The level of all investigated immunoglobulins in the blood serum in patients with splenectomy before the operation was within the physiological norm. The content of IgA initially and for the entire monitoring period was within physiological values. The authors found that the levels of immunoglobulins G and M in



a b

Figure 6.
(a) The level of immunoglobulin IgA, IgG, and IgM in the blood serum in patients in the dynamics in the postoperative period without splenectomy and (b) the level of immunoglobulin IgA, IgG, and IgM in the blood serum in patients in the dynamics in the postoperative period with a splenectomy.

patients with gastric cancer who underwent surgical treatment with splenectomy were reduced almost twofold from the baseline, from the 14th day to the 3rd month of **Figure 6b**.

This fact agrees with the data obtained by our study. Change in the immunological repertoire of B2 cell antigens, weak expression of the CD21 membrane antigen, and a significant amount of CD23+ cells in the case of realization of the main pathway of development of antibody producers in response to thymus-dependent antigens may lead to decrease in antibody production.

Among peripheral blood lymphocytes, the presence of CD19+CD5+ B cells (B1a cells), some of which express the activation antigens CD38 and CD23, is found; a small part of CD5+ B cells is CD25+CD38−. Given the membrane immunophenotype circulating in the peripheral blood of B cells, patients with gastric cancer probably have an alternative TH2 response to pathogens.

In the group of patients after surgical intervention in the volume of gastrectomy with standard D2 lymphodissection and splenectomy, the relative total number of CD19+ B lymphocytes and the number of CD19+CD21+ B cells decreased (the differences are close to reliable) compared to preoperative values. In the group of patients with standard D2 lymphodissection and splenectomy, the percentage of CD5+ B lymphocytes significantly increased from 12.9 to 21.8%, after the operation. Given data on B1 and BMZ populations, this can lead to a weakening of both general and antitumor immunity. Since maintaining the population B1 population is very slow, and the renewal of the BMZ pool is possible only in the spleen.

5. Conclusion

Immunosuppression in patients who underwent surgery (including splenectomy) develops as a result of a disruption in the composition of B cell link. Disorders of the immune response primarily affect the population of B1a lymphocytes, which provides a response to thymus-independent antigens of the second type. Patients in the experimental group may experience decreased production of antibodies, including IgM, which plays an important role in inducing apoptosis of tumor cells.

Conflict of interest

We hereby inform you that there is no conflict of interest.

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