ORIGINAL RESEARCH

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MIGRATION ACTIVITY OF IMMUNOCOMPETENT CELLS WITH DIFFERENT GASTROINTESTINAL TRACT PATHOLOGIES

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This study focuses on the migration and functional activity of immunocompetent cells in the intestinal contents in people with various pathologies of the gastrointestinal tract and in practically healthy people. Actively phagocytic neutrophilic granulocytes and monocytes, as well as lymphocytes without signs of degeneration and plasma cells, were recorded in the intestinal contents. Differences in the migration activity of cells depended on the pathology of the gastrointestinal tract. The levels of immunocompetent cells in the contents of the intestine in practically healthy people were determined. In gastrointestinal diseases, abnormal concentrations of pro-inflammatory cytokines are detected at a high frequency, the highest content of these cytokines have been recorded in malignant intestinal neoplasms.

Objective is to identify the features of the migration and functional activity of immunocompetent cells of the intestinal contents in norm and gastrointestinal pathology.

Keywords: local immunity, mucous membrane, lymphocyte, neutrophilic granulocyte.

The gut-associated lymphoid tissue (GALT) is the largest organ of the lymphatic system and contains all the cells involved in immune response. The lymphoid tissue of the gastrointestinal tract is represented by individual cellular elements (intraepithelial lymphocytes, plasma cells, macrophages, mast cells and granulocytes) and organized structures (Peyer's plaques, appendix, tonsils and lymph nodes). However, the presence and functional activity of immunocompetent cells in the intestinal contents remains unclear. The participation of lymphatic formations in the functional activity of the gastrointestinal tract is predetermined phylogenetically since, throughout the evolution of vertebrates, lymphatic tissue is associated with the digestive canal. Lymphocytes enter the intestinal lumen during digestion [4]. However, there is no cytogram data on the structure of intestinal contents and the presence of functionally active white blood cells. There is also no information about comparative levels of migration and changes in haemogram indicators in normal and pathological conditions. The body normally produces IgA for intestinal microbes and most bacteria in the stool are covered in IgA, but there is little evidence that IgA prevents normal bacteria from entering tissues. To date, IgA secretion remains controversial; it is not fully understood whether IgA is redistributed only by migration from the peripheral blood to the mucosa or can be secreted locally. The output of cells into the intestinal lumen is influenced by factors including the state of the epithelium, the presence of adhesive molecules and the cohesion of epithelial cells. Violations of intercellular contacts and intestinal permeability are found in pathological conditions; instability of intercellular contacts can determine a predisposition for tumour transformation. Tumour cells form weaker bonds with each other than normal tissues, although some transformed tissues retain the ability for contact inhibition [4].

In this regard, this study addressed the activity of local immune responses, the activity of leukocyte migration and lymphocyte recycling in people with various gastrointestinal tract pathologies including inflammatory (colitis, gastroduodenitis), autoimmune (Crohn's colitis) and tumour (rectal cancer, colon cancer, stomach cancer) processes.

Materials and methods. In total, 581 people with chronic gastrointestinal tract pathologies who applied to the medical company «Biocor» were examined, including 106 people with Crohn's colitis, 135 people with non-specific colitis, 123 people with gastroduodenitis, 105 people with colon cancer and 112 people with rectal cancer. As a comparison group, 154 otherwise healthy people living in the city of Arkhangelsk, Russia were examined. The study consisted of peripheral venous blood, mucosal discharge (intestines) and faeces. Citogramma and phagocytosis were studied in smears gram-stained by Romanovsky-Giemsa; the calculation was made at the rate of 100 cells. The expression of markers for T-helper cells and NK cells was determined using indirect immunoperoxidase reaction on lymphocytes. The serum content of cancer markers REA, CA19-9, CA72-4 (CanAg Diagnostica, Sweden), cytokines IL-6, IL-4, TNF-α, IL-10 (Bender MedSystems, Austria), IgA and IgE (Seramun Diagnostica GmbH (Germany), Monobind Inc. (USA)) was determined by enzyme immunoassay on the analyser «Multiscan MC» and automatic «Evolis» analyser of the company «Bio-Rad» (USA). Levels of less than 1.2 g/l were considered IgA deficits. For high levels of IgE > 100 IU/ml was taken. Elevated levels of cytokines were considered for IL-1β > 5 pg/ml, IL-4 > 20 pg/ml, IL-6 > 20 pg/ml, TNF-α > 20 pg/ml, IL-10 > 20 pg/ml. The lymphocytogram was studied by the method of I.A. Kassirsky, 1970 [3]. The research results were processed using the Statistica 6 application software package (StatSoft, USA). The Shapiro-Wilka test was used to determine the statistical difference in values. Differences were accepted at a statistical significance of $p < 0.017$.

Results and discussion. Table 1 shows data on the content of the cytogram of intestinal contents of healthy adults. The limits of neutrophils were 7.11–9.93%, lymphocytes were 17.76–22.5%, monocytes were 1.35–2.49% and plasma cells were 4.41–10.86%. That the results suggest that inflammatory, autoimmune processes and oncological diseases cause an increase in the content of neutrophils, monocytes and lymphocytes in the intestinal lumen (Fig. 1-3). The structure of intestinal content cytograms differs depending on the nature of the disease (Table 1). Inflammatory processes (gastroduodenitis, colitis), including autoimmune (Crohn's colitis) and intestinal malignancies, migration and flushing of neutrophils into the intestinal lumen is activated. Neutrophils are

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immunocompetent cells that migrate to the centres of trouble first; they phagocytize, form traps, and secrete all known cytokines [5, 8]. Neutrophil migration is most significant in non-specific colitis (22.84 ± 0.77%). Crohn's autoimmune colitis is characterised by a higher content of monocytes in the cytogram (10.86 ± 1.22%). In intestinal tumours, specific features of the cytogram have not been established. The main part of monocytes is found in tissues, so even minor changes to the content of monocytes in the blood indicate a significant reaction on the part of the monocyte system. Monocytes phagocyte, form traps, and secrete cytokines and colony-stimulating factors [2]. Monocytes migrate to the inflammation area after neutrophil granulocytes to ensure the elimination of decay products. Migration of monocytes to the mucosa and intestinal lumen is associated with a lack of natural killer cells in the blood. The origin of natural killers is not clear; there are cells that do not have T-lymphocyte antigen (CD3-CD16+CD56+). There are also NK cells with the T-lymphocyte antigen, the thymus-dependent NK cells (CD3+CD16+CD56+), involved in specific immune reactions with antibody formation. The most pronounced reaction of monocytes was found in Crohn's colitis, which is associated with a deficiency in the blood of natural killers in 54.72%. The largest group of cells in the intestinal contents were lymphocytes; their levels did not differ depending on the pathology. In patients with Crohn's colitis, the number of plasma cells increases. The presence of a plasma cell reaction in the intestine of patients with Crohn's colitis confirms local antibody synthesis [7].

In the structure of the lymphocytogram, the level of cells with signs of degeneration is minimal and does not exceed 10% of the total content of small lymphocytes

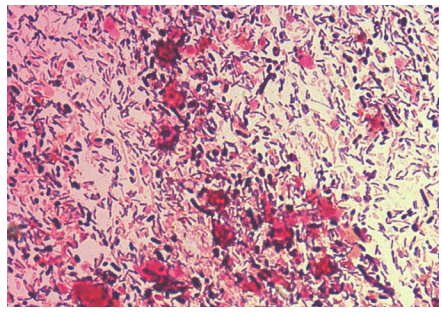

Fig. 1. A smear of feces at nonspecific colitis. Gram stain × 1000. Migration of cells, mainly neutrophils, phagocytosis

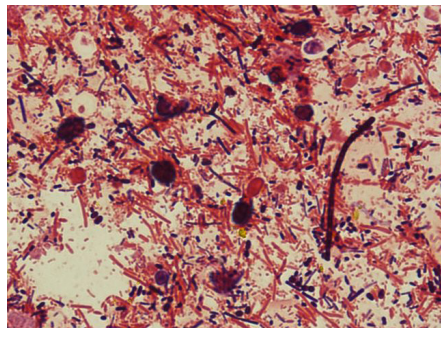

Fig. 2. A smear of feces at nonspecific colitis. Gram stain × 1000. Lymphocytes, plasma cells

in the intestinal lumen; however, the frequency of detecting medium-sized lymphocytes with signs of degeneration is 3-3.5 times higher (24–42%). Migration of lymphocytes is not unilateral; lymphocytes, unlike other immunocompetent cells, are capable of recycling [12, 13, 19]. Among lymphocytes in the composition of the lymphocytogram, 17.46–20.17% are small lymphocytes, which reflect the processes of recycling these cells from tissues to lymphoid organs [12, 13, 19]. Most intraepithelial lymphocytes were identified as CD3+ mature T cells,

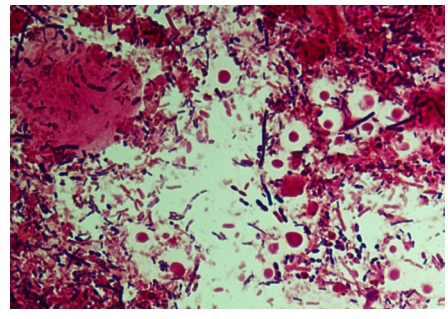

Fig. 3. A smear of feces at Crohn's colitis. Gram stain × 1000. Plasma cells, sorption activity of the epithelium

but 5% and 15% of patients had CD4 or CD8 on the membrane, respectively; the predominance of CD8+ is characteristic of the lamina propria lymphoid pool. Intraepithelial lymphocytes exhibit cytotoxic effects, secrete lymphokines, regulate the regeneration of the mucosal epithelium, and ensure tolerance to food antigens [1]. Almost 50% of the population of intraepithelial lymphocytes consists of regulatory γδT-lymphocytes [14]. A large population of intraepithelial lymphocytes is CD4+CD25+; however, it has been previously demonstrated that they are not capable of proliferation in response to stimulation by a thymus-dependent antigen in vitro [16]. Intraepithelial lymphocytes have receptors for IL-2 but do not secrete it; they instead produce proinflammatory cytokine IL-10 and transforming growth factor-β in large amounts [11]. Lymphocytes CD4+CD25+ participate in tolerance when low doses of protein are administered but do not have a significant effect at high doses [9, 10]. It can be assumed that small lymphocytes, performing an informational role, migrate from the nearest lymphatic formation. In response to the migration of lymphocytes from the intestine to Peyer's plaques,

Table 1

Cytograms of intestinal contents (%)

index	apparently healthy (n=154)	Gastroduodenitis (n=123)	Colitis (n=135)	Colitis Crohn (n=106)	Colon cancer (n=105)	Rectal cancer (n=112)
Neutrophilic granulocytes	8.52±0.47	12.72±0.31	22.84±0.77	13.52±0.19	11.06±0.28	12.2±0.30
Monocytes / macrophages	1.92±0.19	7.58±1.02	6.75±0.85	10.86±1.22	8.81±1.33	8.79±1.32
Lymphocytes	20.13±0.79	28.35±0.58	24.94±0.43	34.36±0.96	32.99±0.96	33.71±0.82
Small lymphocytes	7.09±0.11	17.46±0.34	17.49±0.52	16.41±0.23	17.46±0.36	20.17±0.48
Medium Lymphocytes	13.04±0.23	11.39±0.37	6.62±0.18	17.87±0.36	15.45±0.48	12.4±0.43
Plasma cells	7.50±1.12	5.06±2.41	3.89±2.50	12.95±3.07	7.36±2.83	5.25±2.79
Sorption activity of epithelial cells	58.39±7.39	87.5±2.31	95.32±2.59	126.53±4.73	90.09±3.05	114±4.86
% active phagocytes	49.96±1.21	52.93±0.47	52.42±0.41	58.02±0.09	54.99±0.43	52.12±0.40
Phagocytosis deficiency	47.83	42.28	50.37	50.94	51.43	61.61

mass death of large lymphocytes in the centres of reproduction of lymphoid formation has been observed (6, 17, 20). This, in our opinion, indicates the involvement of these reactions in the formation of tolerance to food antigens and products of the intestinal microflora.

Cytokines are powerful regulators of cell migration and recycling. In healthy people, the frequency of elevated concentrations of pro-inflammatory cytokines in the blood was low, ranging from 1.19–8.43%. IgA deficiency in the blood was found to be within 14.55% and elevated IgE concentrations were 21.59%. In diseases of the gastrointestinal tract, the level of detection of abnormally high reactions from the studied immunological parameters increases; the highest concentrations of proinflammatory cytokines appeared in intestinal malignancies (Table 2).

The highest levels of elevated IL-1β (59.05%) and IL-10 (23.81%) were found in patients with colon cancer. In patients with Crohn's colitis, the highest concentrations of IL-4 in venous blood was 26.32 ± 0.14 pg/ml; elevated levels were observed in 23.58% of the patients. Patients with Chron's colitis also presented the lowest levels of IL-1β (4.58 ± 0.08 pg/ml) with a frequency of elevated concentrations at 36.9%. In non-specific colitis, the cytokine reaction is characterised by a moderate increase in pro-inflammatory cytokines against the background of low levels of anti-inflammatory IL-10, whose content in peripheral blood was 11.32 ± 0.07 PG/ml. The anti-inflammatory cytokine IL-10 causes a decrease in the expression of activating genes and a reduction in the number of receptor structures [15]. In Crohn's colitis and malignancies, elevated IgE concentrations were most often detected (62.19% and 55.24%, respectively). The highest concentrations of reagins (169.71 ± 0.56 IU/ml) were observed in malignant neoplasms. Serum IgA deficiency was reported in 65.71% of patients with rectal cancer. In non-specific colitis, IgA deficiency was detected in 31.71% of the examined patients, twice as often as in healthy patients. Cancer patients presented a high frequency of natural killer (NK) and T-helper (CD4+) deficits, respectively; T-helper deficits were registered in 74.29% of cancer cases and natural killer deficits were registered in 60.71% of the examined cancer patients.

Conclusion. In the composition of the intestinal contents, actively phagocytic neutrophilic granulocytes and monocytes, lymphocytes without signs of degeneration and plasma cells were registered. Neutrophil granulocytes in the intestinal lumen actively phagocytize (49.96 ± 1.21 – 58.02 ± 0.09%), phagocytic activity of neutrophil granulocytes in gastrointestinal pathology is noticeably higher than in healthy individuals. The exception is cases of malignant neoplasms when the deficit of phagocytic activity of neutrophils reached 61.61%. The cytokine reaction in inflammatory processes of the gastrointestinal tract is moderate and relatively uniform compared to that in autoimmune inflammation and tumours.

The most pronounced reaction of monocytes was found in patients with Crohn's colitis; this was associated with a deficiency in the blood of natural killer cells in 54.72% of examined patients. Perhaps the most pronounced migration of monocytes into the intestinal lumen in Crohn's colitis is related to the local variant of antibody formation and the appearance of plasma cells. Eviction of lymphocytes in the lumen of the intestine increases in the pathology of the gastrointestinal tract and reflects the activity of local immune responses. Almost 50% of lymphocytes are represented by medial cells and large lymphocytes; large lymphocytes make up 2–5%. The low level of death of large lymphocytes (1–2%) confirms the development of a local immune response with potential for lymphoproliferation. The features of migration activity in the intestine in malignant neoplasms, compared with other inflammatory processes, could not be identified. The most characteristic features of colon cancer are extremely low phagocytic activity of neutrophil granulocytes in the blood, a high rate of registration of T-helper deficiency (74.29%) and elevated concentrations of IL-10 (23.81%). The highest frequency of elevated blood concentrations of IL-10 was recorded in malignant neoplasms, indicating a significant role of immunosuppression in this pathology.

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Table 2

The frequency of registration of high levels of parameters of the immune defense of people (%)

index	apparently healthy	Gastroduodenitis (n=123)	Colitis (n=135)	Colitis Crohn (n=106)	Colon cancer (n=105)
IL-1ß > 5 pg / ml	1.19		22.96	36.79	59.05
IL-4. > 20 pg / ml	4.34		10.37	23.58	18.09
IL-6 >20 pg / ml	8.43	17.89	14.07	29.25	32.38
TNFα. > 20 pg / ml	5.47		11.85	17.92	20.95
IL-10. > 20 pg / ml	1.14		8.89	16.98	23.81
IgE >100 IU / ml	21.59	41.46	15.56	62.19	55.24
CEA. > 5 pg / ml	4.44	39.84	51.85	36.79	70.48

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