IMMUNOHISTOCHEMICAL ANALYSIS OF LOCAL IMMUNITY IN SALMONELLA AND SHIGELLA INFECTIONS

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The persistent tendency for an increase in the frequency of subacute and chronic forms, and also the existence of a carrier state of the microorganisms responsible for dysentery and salmonella infections are associated with the characteristics of immunity to these infections [3, 4]. It was shown previously that the enzyme status of the immunocytes of the intestinal mucous membrane has characteristic differences both in the dynamics of the acute infectious process and in the chronic course of the disease, and that this can be used as a test for the detection of disturbance of the immune response [1, 2]. However, the study of the cellular enzyme status (CES) does not explain the role of individual lymphocyte subpopulations in the local immune response. Immunohistochemical analysis of cells of the immune system in the mucous membrane of the digestive organs has enabled the mechanisms of disturbances of the immune response to be studied in different infections and approaches to their correction substantiated [5].

The aim of this investigation was an immunohistochemical analysis of the local immune response, compared with the CES of immunocytes in the mucous membrane of the large intestine in different forms of shigella and salmonella infections.

EXPERIMENTAL METHOD

Biopsy specimens of mucous membrane from the large intestine obtained during colonoscopy on 21 patients with dysentery, 5 patients (convalescents) with acute dysentery and 16 with chronic dysentery, and also in 32 patients with salmonella infections: in 14 with the gastrointestinal form (in 7 at the climax of the clinical manifestations, in 7 in the convalescent stage), in 10 patients with a subclinical form, and 1 with a chronic salmonella carrier state. The immunohistochemical studies were carried out on frozen sections (4-5 μ) obtained from biopsy specimens of the mucous membrane of the large intestine with T11, T4, T8 pan-B, and IgD monoclonal antibodies ("Dakopatts," Denmark) by the indirect immunoperoxidase method [9]. The frozen sections were fixed with cold acetone, endogenous peroxidase was inhibited by 0.3% H_2O_2 solution in methanol, and the sections were incubated with monocloncal antibodies for 1 h at room temperature. After incubation with secondary antibodies the mouse immunoglobulins (1:100, "Dekopatts," Denmark) for 45 min at room temperature, the reaction with diaminobenzidine was carried out by the usual method [7]. Some preparations were counterstained with hematoxylin. Standard controls were set up. The preparations were studied under standard magnification (400×) and the ratio of labeled lymphocytes to the total number was expressed as a percentage.

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TABLE 1. Content of Lymphocyte Subpopulations in Mucous Membrane of Large Intestine in Different Forms of Shigella and Salmonella Infections

Group of subjects tested	T11	T4	T8	B_1	I8D
Patients with acute dysentery (convalescence) (n = 5)	++---	++---	+---	++---	+----
Patients with chronic dysentery (n = 16)	+----	+----	+----	+----	+----
Patients with gastrointestinal form of salmonella infection					
At climax of illness (n = 7)	+----	+----	+----	++---	+----
In convalescent stage (n = 7)	+----	++----	+----	+----	+----
Patients with subclinical form of salmonella infection					
Subgroup 1 (n = 5)	++----	+++---	+----	++---	+----
Subgroup 2 (n = 5)	++----	+----	++---	+----	+----
Chronic salmonella carrier (n = 1)	+----	+----	++---	----	----

Legend. -----) from 0 to 5% of lymphocytes; +----) from 10 to 20% of lymphocytes; ++---) from 20 to 40% of lymphocytes; +++--) from 40 to 60% of lymphocytes.

EXPERIMENTAL RESULTS

In acute dysentery in the convalescent stage intensive infiltration of the mucous membrane of the large intestine by lymphocytes was observed (Table 1). B cells predominated, and the helper to suppressor ratio (T4/T8) was shifted toward predominance of $T4^+$-lymphocytes. evidence of marked activation of the cellular and humoral components of immunity. The CES of the immunocytes in this form and stage of the disease showed its highest values.

In chronic dysentery lymphocytic infiltration of the mucous membrane was less marked. The helper to suppressor ratio was close to 1 due to a marked decrease in the number of $T4^+$-lymphocytes (Table 1). The number of B lymphocytes also was reduced, evidence of weakening of humoral immunity and predominance of a cellular suppressor reaction. The parameters of CES of the immunocytes were much lower than in patients with acute dysentery.

At the climax of clinical manifestations of the gastrointestinal form of salmonella infection, the B-lymphocyte population predominated in the infiltrated zone of the mucous membrane of the large intestine. The ratio T4/T8 was close to 1, evidence of activation of the humoral and suppression of the cellular components of immunity. Parameters of CES of the intestinal immunocytes in this stage of salmonella infection were low.

In the convalescent stage of the gastrointestinal form of salmonella infection a tendency was noted for a general decrease in the number of T and B lymphocytes (Table 1). However, the suppressor effect, characteristic of the climax of the clinical manifestations, had disappeared and the T4/T8 ratio was back to normal. The time course of the changes in CES of the immunocytes point to the decline of functional activity in this period of the disease.

Among patients with the subclinical form of salmonella infection, two subgroups were distinguished on the basis of the result of the immunohistochemical investigation. In the patients of subgroup 1 intensive infiltration of the mucous membrane of the large intestine by T and B lymphocytes was observed. The T4/T8 ratio, which was strongly shifted toward predominance of helper T cells against the background of an increase in the number of B lymphocytes, indicated activation of both cellular and humoral components of immunity. In patients of subgroup 2, definite preponderance of suppressor T cells was observed (Table 1) against the background of a general decline in the number of lymphocytes in the mucous membrane of the large intestine, evidence of weakening of the local immune response. A marked suppressor reaction and absence of B lymphocytes were observed in the chronic salmonella carrier. On the basis of the results of the cell enzyme tests patients with the subclinical form also were subdivided into two subgroups. In the patients of subgroup 1, CES of the intestinal immunocytes was close to the parameters of CES in the convalescent stage of the gastrointestinal form, whereas in the patients of subgroup 2 values of CES were much lower and were close to values of CES of chronic salmonella carriers.

Thus both cell enzymo tests and immunohistochemical analysis of biopsy material from the mucous membrane of the large intestine of patients with a subclinical form of salmonella infection yielded results on the basis of which two subgroups of patients with this form could be distinguished. Activation of the cellular and humoral components of immunity discovered in the patients of subgroup 1, and parameters of CES close to their values in the convalescent stage of the gastrointestinal form, meant that the course of the disease in this particular subgroup could be identified as acute. Weakening of the local immune response and parameters of CES close to their values in chronic salmonella carriers, discovered in the patients in subgroup 2, enabled the course of the disease in these patients to be classified as "chronic." Heterogeneity of the immune mechanisms in patients with the subclinical form of salmonella infection may be due both to the properties of the antigen, its dose, and the rhythm of its entry into the body, and with the particular features of the immune response, controlled by genes of the major histocompatibility complex [8].

The immunohistochemical analysis, conducted on patients with chronic forms of dysentery, and also in patients with the subclinical form of salmonella infection in subgroup 2 and the chronic salmonella carrier, revealed the general pattern of local immune reactions connected with an increase in the $T8^+$-subpopulation of lymphocytes. The marked suppressor reaction is evidently an important pathogenetic step in the process of chronic transformation.

The immunohistochemical study of biopsy material from patients with an acute form of dysentery and salmonella infection revealed activation of local immunity. However, in the stage of convalescence from acute dysentery, the local immune response was distinct due to activation of both T-cell and B-cell components of immunity, whereas in the gastrointestinal form of salmonella infection (climax of clinical manifestations) it was mainly on account of the B-cell component. These features of the immune response are evidently determined by different antigenic properties of shigellas and salmonellas [6]. Parameters of CES of immunocytes in the mucous membrane of the large intestine in patients with a chronic type of infection reached the highest values.

Immunohistochemical investigation of biopsy specimens of the mucous membrane of the large intestine thus led to the discovery of the different kinds of local immunity observed in dysentery and salmonella infections, which correlate clearly with parameters of CES of the immunocytes both in the mucous membrane of the large intestine and in the peripheral blood [3, 4]. Consequently, taking into account the results of the immunohistochemical study, parameters of CES can be used both for prognosis and for immunocorrective therapy.

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