Antibodies to the Glycoprotein of *Bacillus megatherium* H in Patients with Oncological and Nononcological Pathologies of the Alimentary Canal

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A high level of antibodies to the glycoprotein fraction of *Bac. megatherium H* with a molecular weight of 8-10 kD is demonstrated in stomach cancer; in highly differentiated stomach cancer the level of antibodies to the glycoprotein with a molecular weight of 12-15 kD is also increased. The level of antibodies to the glycoprotein with a molecular weight of 10-12 kD is increased in peptic and duodenal ulcer. It is suggested that these glycoprotein fractions are useful for diagnosing oncological and nononcological diseases of the alimentary canal.

Key Words: *antibodies; oncofetal glycoprotein; oncological diseases*

Oncofetal antigens are often used in the immunodiagnostics of tumors. These antigens are expressed by most if not all tumors developing from a common nondifferentiated precursor cell. Tissue expression of an oncofetal antigen provides valuable information regarding the stages of tumor transformation and progression [7]. Autologous tumor vaccine [2] has been employed as a test antigen in the diagnostics of recurrences and metastases in patients with stomach cancer. Lactoferrin, which may serve as a marker of the acinar differentiation of various glandular tumors, has been identified in highly and moderately differentiated adenocarcinomas [4-6]. Previously we showed that the glycoprotein isolated from *Bacillus megatherium* H reacts with antibodies in the presence of lymphoid or epithelial tumors [1,3]. After fractionation of the glycoprotein we obtained 8 fractions differing in molecular weight.

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The aim of this study was to examine the humoral immune response to the *Bac. megatherium* H glycoprotein fractions in oncological and nononcological diseases of the alimentary canal.

MATERIALS AND METHODS

Serum was obtained from 57 patients. Thirty patients were suffering from stomach cancer of stage II-IV or colon cancer of stage II-IV and 10 patients had benign neoplasms (polyps, fibromas, papillomas). These patients were under treatment in the First Municipal Hospital of Novosibirsk. Eleven patients had a history of peptic and duodenal ulcer and were being treated in the Gastroenterological Department of Municipal Hospital № 18 of Novosibirsk. Six sera were obtained from donors aged 24-49 years. Blood from the cancer patients was obtained before surgery. Glycoprotein from *Bac. megatherium* H was fractionated on a Toyopearl HW-55 column (Japan). Fraction 1 (molecular weight 15-18 kD, fraction 2 (12-15 kD), fraction 3 (10-12 kD), fractions 4, 5, and 6 (5-6 kD), fraction 7 (3-4 kD),

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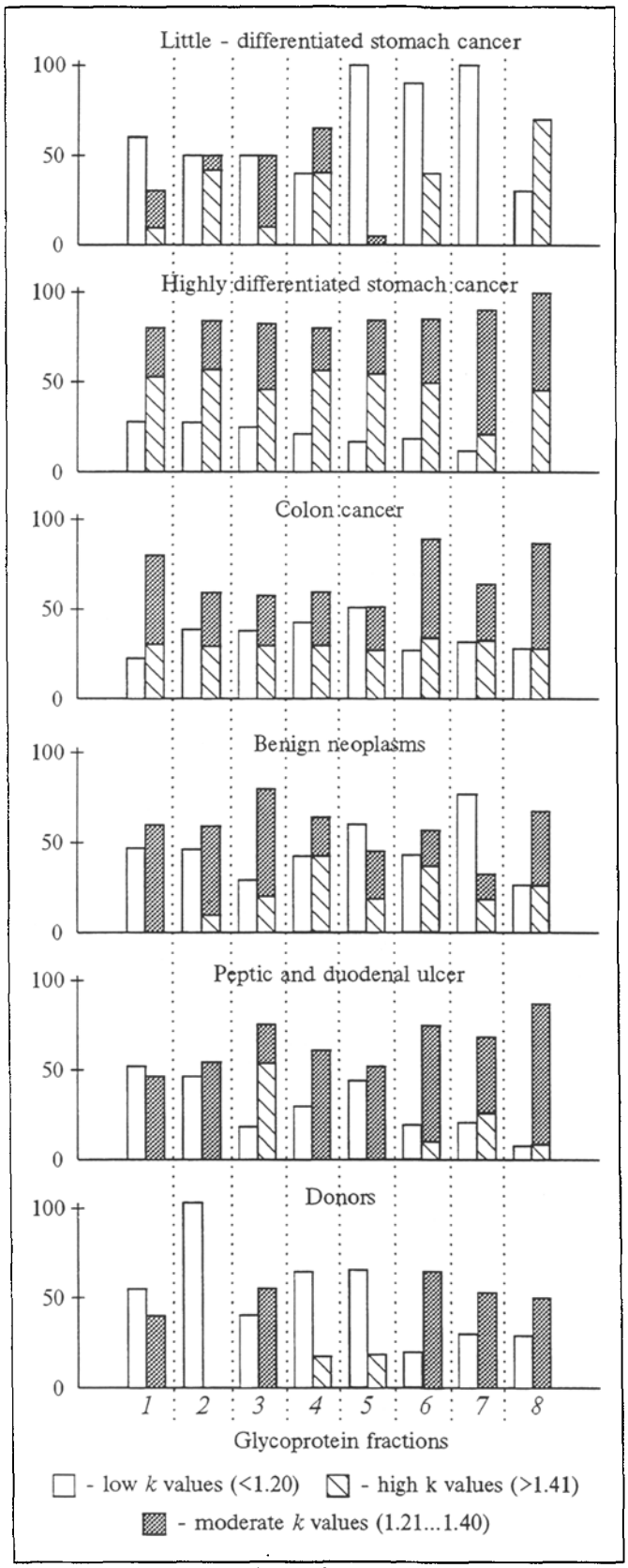

Fig. 1. Distribution of K values in the identification of antibodies to *Bac. megatherium* H glycoprotein fractions in patients with oncological and nononcological diseases of the alimentary canal.

and fraction 8 (8-10 kD) were obtained. Antibodies to the glycoprotein fractions were detected in an immunoenzyme assay using Russian-manufactured microplates (Krasnoyarsk Chemical Plant Enisei). A glycoprotein solution (50 μg/ml, 100 μl) in a carbonate-bicarbonate buffer (pH 9.2) was added to the plates. The plates were then washed with buffered physiological saline (BPS) containing 0.05% Tween-21. Bovine serum albumin (200 μl of a 2% solution in BPS, pH 7.4) was added to each well to prevent nonspecific sorption. The tested serum (100 μl) was added to each well at a 1:50 dilution. Anti-human immunoglobulin antibodies conjugated with horseradish peroxidase (100 μl, 1:1000, N. F. Gamaleya Institute of Epidemiology and Microbiology) were added to BPS (pH 7.4) containing 0.05% Tween-21 and 1% BSA. The plates were incubated at 37°C for 1 h, washed three times with BPS containing Tween-21, and the substrate (100 μl) consisting of orthophenylene diamine (10 μg in 100 μl 20 mM, citrate buffer, pH 4.7, and 10 ml 30% hydrogen peroxide) was added. The reaction was terminated by the addition of 1 M sulfonic acid at the moment when the color appeared. Wells in which one of the reaction components (antibody, serum, conjugate) was omitted served as a control. The reaction was read in an AKI.Ts.01 photometer. The coefficient: $K=D_o/D_c$, where D_o is the absorbance of the well with the tested serum and D_c is the absorbance of the control well, was employed for statistical analysis: K values <1.20 were regarded as low, 1.21-1.40 as moderate, and >1.40 as high.

RESULTS

The cancer patients were divided into 4 groups depending on the localization and differentiation of the pathological process: patients with little-differentiated stomach cancer (LDSC, group I), patients with highly differentiated stomach cancer (HDSC, group II), patients with highly differentiated colon cancer (CC) localized in the sigmoid colon or rectum (group III), and patients with benign neoplasms (BN, group IV).

Analysis of the results revealed differences related to the degree of tumor differentiation. In LDSC, the values of K were statistically different from the control only for fraction 8 of glycoprotein. In HDSC, K was statistically different for all glycoprotein fractions except fractions 3 and 7 (Table 1). In CC, K was lower than in HDSC, but was predominantly higher than in patients with BN and in donors; however, the difference was statistically insignificant. The maximum K

TABLE 1. Mean Values of Coefficient K in Patients with Oncological and Nononcological Diseases and in Donors ($M \pm m$)

Group	K values in identification of antibodies to *Bac. megatherium* H glycoprotein fractions							
	1	2	3	4	5	6	7	8
LDSC ($n=10$)	1.12±0.06	1.29±0.07	1.16±0.06	1.43±0.11	1.02±0.09	1.27±0.09	0.95±0.09	1.46±0.09*
HDSC ($n=12$)	1.46±0.10*	1.56±0.11*	1.37±0.07	1.45±0.05*	1.49±0.09*	1.42±0.06*	1.38±0.07	1.48±0.08*
CC ($n=8$)	1.36±0.08	1.34±0.10	1.31±0.08	1.34±0.10	1.33±0.11	1.48±1.14	1.28±0.06	1.30±0.05
BN ($n=10$)	1.24±0.03	1.26±0.04	1.27±0.05	1.30±0.05	1.23±0.04	1.30±0.06	1.22±0.05	1.31±0.06
PU ($n=11$)	1.23±0.02	1.22±0.02	1.36±0.04*	1.24±0.02	1.27±0.02	1.32±0.03	1.31±0.03	1.33±0.03
Donors ($n=6$)	1.22±0.04	1.14±0.03	1.24±0.04	1.19±0.06	1.19±0.06	1.23±0.06	1.20±0.03	1.20±0.04

Note. Asterisk indicates values significantly different ($p<0.05$) from those in donors.

values for fraction 1 were obtained in patients with HDSC, although the occurrence of positive K values was higher in CC patients predominantly at the expense of moderate K values (62.5%) (Fig. 1). There were no high K values for fraction 1 in patients with BN and peptic ulcer (PU).

The values of K for fraction 2 of the glycoprotein of *Bac. megatherium* H in the group of patients with HDSC were significantly different from those occurring in the group of patients with BN ($p<0.05$) and PU ($p<0.02$). In HDSC, the K values for fraction 2 were high in 75% of cases, whereas in LDSC the occurrence of high K values was 2-fold lower. The values of K for fraction 2 were never high in patients with PU or in donors. The specificity of this test system in revealing the antibodies to fraction 2 of the glycoprotein of *Bac. megatherium* H was 100% in donors, and sensitivity in HDSC patients was 75%.

The occurrence of positive (moderate and high) K values for fraction 3 was high (81.81%) in PU. The values of K were significantly different from those in donors, indicating that fraction 3 of the glycoprotein is diagnostically most valuable in the case of PU.

Coefficient K for fractions 4, 5, and 6 was significantly different only in HDSC patients and donors. For fraction 7 the coefficient was not significantly different in any group. For fraction 8 in HDSC and LDSC it was significantly different from K in the group of donors.

Thus, in patients with stomach cancer the concentration of antibodies to the glycoprotein of *Bac. megatherium* H with a molecular weight of 12-15 kD is increased. In peptic and duodenal ulcer the concentration of antibodies to the glycoprotein with a molecular weight of 10-12 kD is increased. These fractions are promising for the diagnostics of oncological and nononcological diseases of the alimentary canal.

REFERENCES

1. A. I. Autenshlyus, V. A. Semernikov, S. V. Khutornoi, *et al., Byull. Eksp. Biol. Med.*, **111**, № 5, 525 (1991).
2. L. V. Drakina, S. Yu. Fedotov, and I. Ya. Kogan, in: *Immunological Aspects of the Diagnostics of Malignant Neoplasms* [in Russian], Moscow (1988), p. 102.
3. D. G. Zatula and V. A. Semernikov, *Immunology of Cross-Reactive Microbial Antigens and Blastoma Cells* [in Russian], Kiev (1986).
4. G. Barresi, G. Barjoed, A. Krogblahl, and G. Bretlan, *Acta Otolaryng.*, **412**, 130 (1984).
5. G. Caselitz, T. Gaup, and G. Seifert, *Virch. Arch. A*, **394**, 61 (1981).
6. G. Tuccari and G. Barresi, *Ibid.*, **406**, 67 (1985).
7. S. Valean, D. Malide, M. Petresu, and R. Vlaicu, in: *Eleventh Europ. Immunol. Meet., Espoo, June 9-12, 1991, Abstr., Europ. Fed. Immunol. Soc.*, Helsinki (1991), p. 28.