MICROBIOLOGY AND IMMUNOLOGY

Contribution of the Disease Process to Phenotypic Variability of Human Colon Microflora

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Quantitative and qualitative compositions of microbiocenosis in the colon of healthy subjects and patients with various somatic diseases (infectious endocarditis, rheumatic heart disease, chronic renal insufficiency, and acute odontogenic phlegmons) are compared. It is concluded that disease is a powerful factor of the intestinal microbiocenosis variability. No changes in the colonic microflora specific for the diseases are detected.

Key Words: *normal microflora; variability of human microbiological phenotype*

In our previous studies we considered relative contributions of the genotype and environment to individual variability of microbiologic phenotype in the human colon [3,4]. These studies showed that the variability of the qualitative (species) and quantitative compositions of transitory components of intestinal microbiocenosis as well as the content of indigenous microflora are determined by prevailing influence of paratypic variance. The latter involves a variety of effects, including those of endogenous origin, exerted by the host organism.

As known, numerous diseases are caused by microorganisms for which the gastrointestinal mucosa is the portal of entry. It is possible that a disease alters the microflora of a biotope. For example, shifts in colonic microflora occur in certain somatic diseases [1,5,9]. However, the contributions of disease to the variability of the colonic microbial population have not been assessed and require special study. We attempted to analyze qualitative and quantitative compositions of colonic microflora in several communicable and noncommunicable diseases.

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MATERIALS AND METHODS

A total of 127 patients aged 15 to 78 years (including 42.5% of females) treated in clinics of the Sechenov Moscow Medical Academy during 1992-1993 were examined. The colonic microflora was studied prior to antibiotic therapy in order to rule out its effect on the microflora.

Eighteen patients had subacute infectious endocarditis (SIE), 10 of them in the active stage. Eighteen patients had rheumatic heart disease (RHD, remission in 15 patients); 13 patients had various renal diseases in the stage of chronic renal failure (CRF, all the patients were on regular hemodialysis); and 78 patients had acute odontogenic phlegmons (AOP) in the maxillofacial region.

The control group consisted of 50 healthy subjects aged 17 to 75 years (60% of them were women) without clinical signs of dysbacteriosis.

Colonic microflora was examined using the procedure [6]. Microorganisms were identified on the basis of their morphological, cultural, and biochemical properties. Their contents were expressed as lg CFU/g.

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TABLE 1. Qualitative and Quantitative Compositions of Colonic Microbiocenosis in the Healthy Subjects and Patients with Subacute Infectious Endocarditis (SIE), Rheumatic Heart Disease (RHD), Chronic Renal Failure (CRF), and Acute Odontogenic Phlegmons (AOP)

Organisms	Healthy subjects		SIE		RHD		CRF		AOP	
	%	lg CFU/g	%	lg CFU/g	%	lg CFU/g	%	lg CFU/g	%	lg CFU/g
Genera:										
Bifidumbacterium	100.0	8.66±0.25	100.0	8.39±0.38	100.0	8.89±0.36	100.0	8.69±0.38	85.9	6.67±0.35
Lactobacillus	100.0	6.53±0.15	100.0	6.68±0.48	100.0	8.39±0.36	100.0	7.44±0.65	91.0	5.35±0.37
Enterococcus	100.0	7.20±0.34	100.0	5.34±0.85	100.0	5.97±0.71	100.0	5.75±0.96	89.7	5.74±0.40
Escherichia	100.0	7.82±0.24	100.0	7.75±0.37	100.0	7.70±0.82	100.0	8.53±0.52	100.0	8.23±0.12
Staphylococcus	96.0	6.05±0.35	83.3	4.62±0.60	55.6	2.98±0.72	84.6	4.78±0.73	55.1	3.24±0.37
Candida	82.0	5.17±0.42	50.0	2.94±0.75	38.9	1.89±0.66	30.8	1.68±0.80	50.0	2.73±0.33
Clostridium	72.0	4.48±0.44	44.4	1.89±0.62	44.4	2.13±0.65	38.5	1.66±0.69	37.2	1.79±0.29
Citrobacter	56.0	1.17±0.55	22.2	1.79±0.32	27.8	1.21±0.53	23.1	1.35±0.75	15.8	0.68±0.28
Enterobacter	24.0	1.11±0.32	33.3	1.27±0.49	5.6	0.17±0.17	23.1	1.25±0.70	7.7	0.31±0.19
Klebsiella	32.0	0.99±0.27	33.3	1.74±0.68	33.3	1.47±0.68	15.4	1.03±0.73	33.3	2.04±0.42
Proteus	16.0	0.48±0.19	0.0	0.00	11.1	0.58±0.40	23.1	2.25±0.95	17.9	0.87±0.22
Species:										
E. coli with normal enzymatic properties	96.0	7.45±0.33	100.0	6.49±0.64	88.9	6.86±0.64	100.0	8.45±0.25	98.6	7.73±0.25
E. coli with weakened enzymatic properties	18.0	1.26±0.38	27.8	1.80±0.76	61.1	4.10±0.87	15.4	0.53±0.38	42.3	2.78±0.40
Lactose-negative *E. coli*	16.0	0.86±0.33	55.6	3.53±0.85	44.4	2.90±0.85	61.5	3.85±1.00	32.1	2.08±0.36
S. aureus	22.0	1.41±0.40	44.4	2.16±0.64	22.2	1.24±0.60	23.1	1.15±0.63	20.5	1.15±0.27
S. epidermidis and *S. saprophyticus*	76.0	4.32±0.47	72.2	3.76±0.72	33.3	1.74±0.65	84.6	4.63±0.74	34.6	2.10±0.35

The data were subjected to standard statistical treatment using comparative analysis and analysis of variance (ANOVA) [8].

RESULTS

Quantitative and qualitative compositions of colonic microbiocenosis in the patients and healthy subjects are shown in Table 1. Differences in colonic microflora between the patients and healthy subjects as well as between the patient groups are described in detail below.

In feces of patients with SIE, organisms from the genera *Citrobacter*, *Clostridium*, and *Candida* were found much more frequently than in feces of controls, whereas lactose-negative *Escherichia coli* were found 3.5 times more frequently ($p<0.01$). The total content of *Staphylococcus*, *Clostridium*, and *Candida* organisms in the colon of these patients was significantly lower than in the control group.

In feces of patients with RHD, *Staphylococcus*, *Clostridium*, *Citrobacter*, and *Candida* were present in markedly smaller numbers than in those of healthy subjects. In particular, nonpathogenic staphylococci (*S. epidermidis* and *S. saprophyticus*) could be cultured from feces of only 33% of the patients, i.e., were encountered 2.8 times less frequently than in feces of the controls ($p<0.01$), whereas lactose-negative *Escherichia coli* were present in the feces of these patients much more frequently (by 2.8 times, $p< 0.05$), as were *E. coli* with weakened enzyme activity (by 3.4 times, $p<0.01$). Colonic microbiocenoses of rheumatic patients also contained much more lactobacilli and *E. coli* with altered enzyme activity. Fecal concentrations of pathogenic *Staphylococci*, *Clostridia*, *Enterobacter*, and *Candidae* and nonpathogenic *Staphylococci* were significantly lower ($p<0.05$).

The occurrence of *Clostridia*, *Candidae*, and *Citrobacter* was low in patients with CRF on regular hemodialysis, while the occurrence of lactose-negative *E. coli* was 3.8 times as high as that as in the controls ($p<0.01$). *Candidae* and *Clostridia* occurred in feces of patients with CRF in markedly lower numbers than in those of controls ($p<0.01$), while *Protei* were present in fairly large numbers.

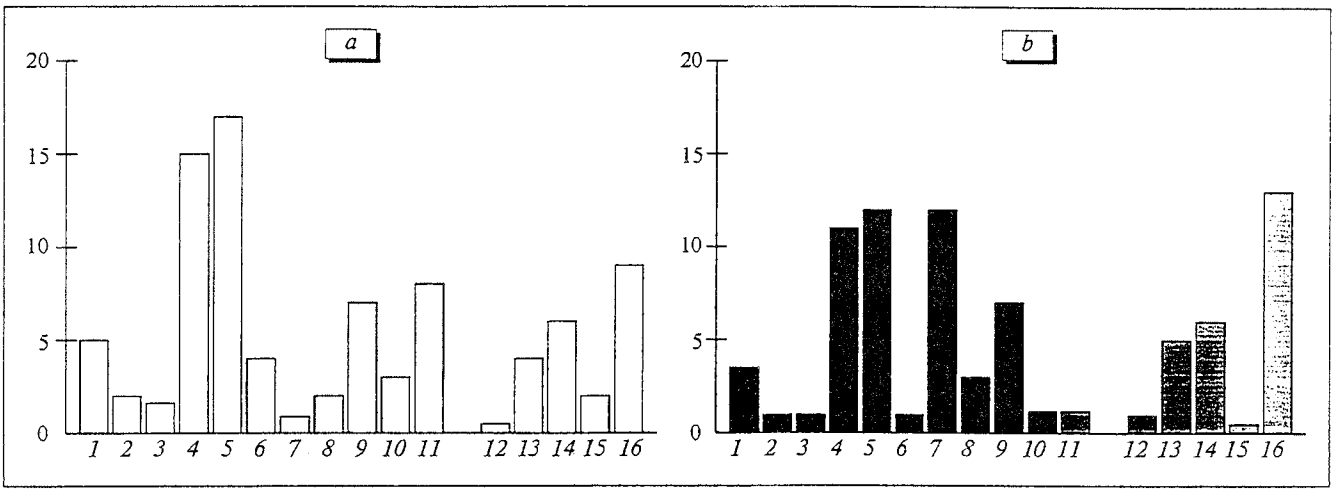

Fig. 1. Contribution of deviations from normal in the state of human health on the interindividual diversity of colonic microbiocenosis. Here and in Fig. 2: *a*) plating capacity, %; *b*) content, lg CFU/g. Ordinate: contribution, %. Identified components of microbiocenosis here and in Fig. 2: *1-11*) microbial genera, including *Bifidumbacterium* (*1*), *Lactobacillus* (*2*), *Enterococcus* (*3*), *Escherichia* (*4*), *Candida* (*5*), *Clostridium* (*6*), *Citrobacter* (*7*), *Enterobacter* (*8*), *Staphylococcus* (*9*), *Klebsiella* (*10*), and *Proteus* (*11*); *12-16*) microbial species, including *E. coli* with normal (*12*) and weakened (*13*) enzyme activity, lactose-negative *E. coli* (*14*), *S. aureus* (*15*), and nonpathogenic staphylococci (*S. epidermidis* and *S. saprophyticus*, *16*).

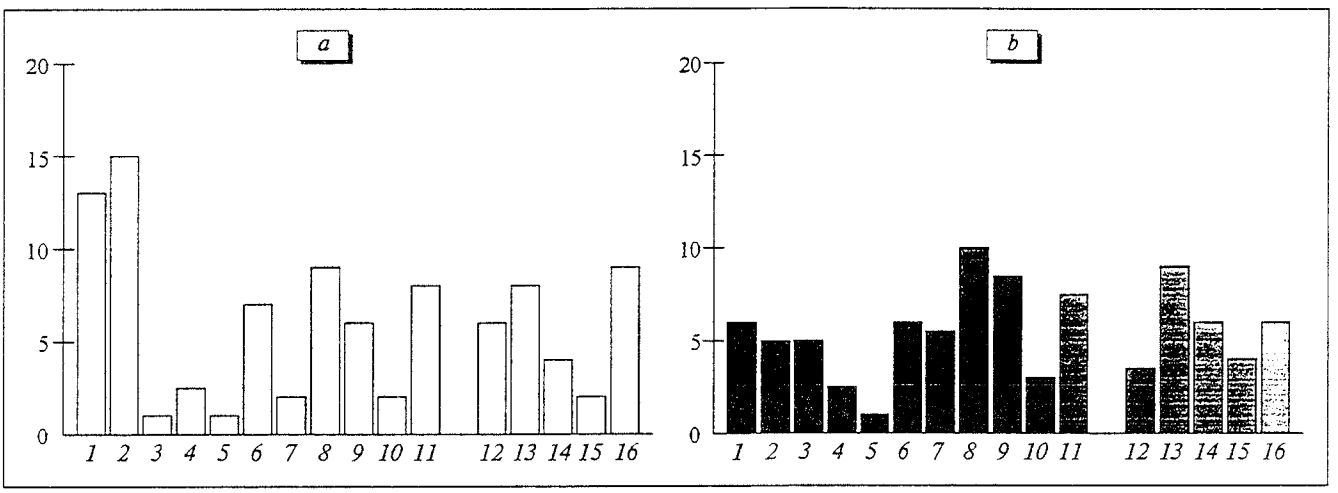

Fig. 2. Effect of the type of disease process on the variability of colonic microbiocenosis.

Greater differences from the control were found in patients with AOP. Some of these patients were deficient in indigenous organisms such as bifidobacteria, enterococci, and lactobacteria, and contained greater numbers of *E. coli* with modified enzyme activity. Nearly all other members of the intestinal microbiocenosis occurred in these patients less frequently and in lower concentrations than in healthy subjects.

Comparison of our data revealed two characteristics shared by colonic microflora in all studied diseases. First, *Clostridia*, *Citrobacter*, and *Candidae* were present in feces of the patients less frequently and lactose-negative *E. coli* much more frequently than in feces of healthy subjects. Second, staphylococci were contained in reduced concentrations in the feces of all patients. Of interest in this connection are the results of ANOVA which showed that the presence of a disease process of whatever type is an important cause of deviations from normal in the microbiological phenotype of patients (Fig. 1).

On the other hand, in each disease the microflora was found to have some distinctive features. Thus, *S. aureus* and *Enterobacter* were encountered most frequently in patients with SIE; *Klebsiellae* were rarely found in patients with CRF, while *Protei* were found in them more frequently than any other organisms; *Enterobacter* and nonpathogenic *Staphylococci* were detected less frequently than any organisms in the groups with RHD and AOP, whereas *E. coli* with weakened enzyme activity were encountered rather frequently in these groups. Low

numbers of bifidobacteria and lactobacilli appear to be characteristic of the colonic microflora in patients with AOP.

Although the intergroup differences described above were obvious visually, the ANOVA failed to demonstrate their discriminatory significance. Calculations showed (Fig. 2) that the nature of disease could not be the endogenous factor determining the specificity of colonic microbiocenosis in the patients. This implies that the internosological vector of variability of the colonic microbiological phenotype in the human body makes a much less significant contribution to the variability of microbial ecology than does the physiological condition.

This study demonstrated a relationship between colonic microbial ecology and human health. Our findings led us to conclude that disease processes themselves should be classed among the powerful endogenous determinants of variability of the microbiological phenotype of the human colon [6,7]. However, alterations in the microbial ecology of this biotope occurring in various diseases cannot be called specific because most of them are of the same type and differ only in degree. This conclusion is in accord with the results reported previously [2]. Since the role and place of normal flora in the pathogenesis of diseases considered here have not been defined, it seems plausible that the imbalance of microbial ecology is a consequence of the underlying disease process and reflects the extent to which homeostasis is disturbed in the host.

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