HETEROGENEIC ANTIGENS OF GENETIC RECOMBINANTS OF Escherichia coli SIMILAR TO HUMAN A, B, AND O ANTIGENS

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Genetic crosses were carried out between donor strains of Escherichia coli of pathogenic serotypes containing heterogeneic antigens and recipient strains of E. coli K-12 not possessing these antigens. The presence of heterogeneic antigens as unselective characters was determined in the various groups of recombinants obtained. The results indicate closer linkage of the locus of the heterogeneic type B human antigen with the locus of the his^+ gene.

In 1945, Zhukov-Verezhnikov postulated that the presence of a human antigen in pathogenic bacteria may cause an increase in their virulence [4] and interfere with the development of immunity after vaccination [5]. The presence of heterogeneic antigens common to both man and bacteria was subsequently demonstrated, and this phenomenon has been termed biological or antigenic mimicry.

Studies of enteropathogenic strains of Escherichia, belonging to different serological groups, has shown that they possess heterogeneic antigens similar to human isoantigens of the ABO type [1, 3, 6-8].

The object of the present investigation was to study the possibility of transmission of genetic determinants controlling the synthesis of heterogeneic antigens from bacteria of pathogenic serotypes to untyped strains of E. coli and to determine the localization of these determinants on the chromosome.

EXPERIMENTAL METHOD

Strains of E. coli 1-O55, 2-O55, and 3-O55, possessing a human type O (H) heterogeneic antigen, and strains of E. coli H-35 of serotype 086 : K- : H25, possessing a human type B heterogeneic antigen, were used as donors. These strains were obtained from the department of microbiology, Kalinin Medical Institute, and from the collection of the All-Union Escherichia Center. After treatment of the bacterial population of strain H-35 with nitrosoguanidine, its auxotrophic variant (lac^- cys^-) was obtained, and from this, in turn, F' lac^+ donors were obtained by introduction of the F' lac^+ factor from E. coli strain K-12 (200PS).

Standard polyauxotrophic strains E. coli K-12 : C600 (thr^- leu^- B' lac^- S^r) and AB1157 (thr^- leu^- pro^- his^- arg^- lac^- S^r) were used as the recipients.

Crossing was carried out by mixing 18-h broth cultures of donors and recipients in equal volumes (0.5 ml) in 6 ml fresh nutrient broth. The mixtures were incubated for 20 h at 37°C, after which the bacteria were sedimented by centrifugation. After resuspension in 0.5 ml physiological saline the bacteria were seeded on dishes with medium enabling genetic recombinants of the appropriate type to be selected.

To detect antigens of the human ABO system in the bacteria a modified technique of adsorption of specific isoagglutinins by heterogeneic bacterial antigens [2] was used.

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TABLE 1. Results of a Study of Recombinants from Crosses of Donor Strains of E. coli Possessing Heterogeneic Antigens Similar to Antigens of the Human ABO System and Recipient Strains of E. coli K-12

Donor strain of E. coli			Recipient strain of E. coli K-12	Selective markers									
				thr+ leu+		pro+		his+		arg+		lac+	
designation	antigenic characteristics	Presence of heterogeneic antigens		number of recombinants studied	number containing heterogeneic antigen	number of recombinants studied	number containing heterogeneic antigen	number of recombinants studied	number containing heterogeneic antigen	number of recombinants studied	number containing heterogeneic antigen	number of recombinants studied	number containing heterogeneic antigen
H=35	O86:K:H25	B	AB1157	10	4	10	3	10	8	10	5	10	3
			C600	8	1								
1=O55	O55	O (H)	AB1157	17	3	12	2	16	14	18	4	2	2
			C600	20	6								
2=O55	O55	O (H)	C600	20	12								
3=O55	O55	O (H)	C600	10	6								

TABLE 2. Results of Castellani's Adsorption Test on Serum against E. coli O55 with Cultures of E. coli Containing and not Containing Human Type O(H) Heterogeneic Antigen

Serum against E. coli O55 (strains used for adsorption)	Strains of E. coli used as antigen [contain heterogeneic human type O(H) antigen]					
	1-O55	P1	P2	P3	P4	P5
Unadsorbed	1:3200	1:3200	1:3200	1:3200	1:3200	1:3200
Strain 1-O55 contains heterogeneic O(H) antigen	—	—	—	—	—	—
Recombinant strain [P1 contains heterogeneic O(H) antigen]	—	—	—	—	—	—
Strain AB1157 [does not contain heterogeneic O(H) antigen]	1:3200	1:3200	1:3200	1:3200	1:3200	1:3200
Recombinant strain P6 [does not contain heterogeneic O(H) antigen]	1:3200	1:3200	1:3200	1:3200	1:3200	1:3200

EXPERIMENTAL RESULTS

Genetic recombinants selected for individual genetic markers were subcultured on an identical selective medium and tested for the presence of heterogeneic B or O(H) antigen as unselective markers. As Table 1 shows, many genetic recombinants inherited the genetic determinants of these antigens. When strain H-35 was used,the heterogeneic antigen was detected most frequently among the his^+-recombinants. This suggested that the genes responsible for synthesis of the type B heterogeneic antigen are more closely linked with his^+ locus. This hypothesis was confirmed in a supplementary experiment to study the various types of recombinants obtained as the result of 2-h crosses of the bacteria of this strain with AB1157 recipient cells. During the analysis of these recombinants the heterogeneic antigen was discovered only in the his^+ type. The extremely low frequency of appearance of recombinants in analogous 2-h crosses of strains 1-O55, 2-O55, and 3-O55 prevented similar investigations from being undertaken with respect to the O(H) heterogeneic antigen.

It is interesting to note that the localization of the genetic determinants of the heterogeneic Forsmann antigen in Salmonella also is linked with the locus of the his^+ gene [9].

In the experiments of series II, recombinants obtained by crossing strains 1-O55 and AB1157 were studied in the agglutination test with OB serum against E. coli O55. As Table 2 shows, recombinants (P1-P5) possessing heterogeneic antigen gave agglutination in the same titer as the original strain 1-O55, whereas recombinants not containing the heterogeneic antigen (P6-P10) behaved similarly to strain AB1157, i.e., they were agglutinated spontaneously in physiological saline.

The reaction of adsorption of serum O55 by different bacteria, carried out by Castellani's method, showed that the serum, adsorbed by the original strain 1-O55, just as also by the recombinants containing antigen, lost its agglutinable properties in relation both to the strain used for adsorption and all the other strains of that group. Adsorption of the O-55 serum by cells of E. coli AB1157, and also by P6-P10 recombinants not containing heterogeneic antigen had no effect on the titer of the adsorbed serum.

The results indicate the possibility of transmission of heterogeneic antigens from bacteria of typed strains to bacteria of untyped strains of E. coli. Meanwhile, genetic analysis of the recombinants suggests that the genetic determinants controlling synthesis of the human type B heterogeneic antigen are located close to the his^+ locus on the chromosome of E. coli.

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