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GENETIC ANALYSIS OF THE O ANTIGENS IN *SALMONELLA* I. HEREDITY OF THE BLOOD GROUP-ACTIVE O ANTIGENS IN *SALMONELLA* GROUPS G, R, AND U

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It is clarified that there are common antigenicities between *Salmonella* O antigens and human blood group antigens (Iseki 1952; Iseki 1956; Springer 1970), for examples, between O antigen 5 of *Salmonella* group B and blood group A-active Forssman antigen F_A, O antigen 13 of *Salmonella* group G and blood group antigen H, O antigen 40 of *Salmonella* group R and blood group antigen A, and O antigen 43 of *Salmonella* group U and blood group antigen B.

In the present paper, the genetic determination of the blood group-active O antigens of *Salmonella* was studied by means of Hfr or F′ crosses between strains of group B (O antigens 1, 4, 12 or 1, 4, 5, 12) and G (O antigens 13, 22 or 13, 23), R (O antigen 40) or U (O antigen 43), and the manifestation mechanism of phage-mediated O antigen 1 in *Salmonella* groups A, B and D was also examined. Some of the results have appeared in a preliminary form (Iseki and Kishi 1968).

MATERIALS AND METHODS

Bacterial strains: Group B bacteria: SW 1391 *Salmonella abony* (1, 4, 5, 12) Hfr *aro*$^-$ *met*$^-$ *str-r* (Mäkelä 1963), which was kindly given to us by Dr. T. Iino (National Institute of Genetics, Japan); S-3 F′ *S. paratyphi* B 8006 (4, 5, 12) F′·lac (originally from *Escherichia coli* K12 W 3747) prototroph *str-s*; SK-83 *S. paratyphi* B var. *odense* (1, 4, 12) F$^-$ *his*$^-$ *str-r*. Group D bacterium: S-57 F′ *S. typhi* H901W (9, 12) F′·lac *ser*$^-$ *str-s*: Group G bacteria: SK-gr F′ *S. grumpensis* (13, 23) F′·lac *trp*$^-$ *str-s*; SK-po 01^r *S. poona* (13, 22) F$^-$ *his*$^-$ *str-r*. Group R bacteria: SK-rio F′ *S. riogrande* (40) F′·lac *gly*$^-$ *str-s*; SK-rio 01^r *S. riogrande* (40) F$^-$ *his*$^-$ *str-r*. Group U bacteria: SK-mil F′ *S. milwaukee* (43) F′·lac *arg*$^-$ *str-s*; SK-mil 01^r *S. milwaukee* (43) F$^-$ *his*$^-$ *str-r*.

Isolation of auxotrophic mutants. Mutants in these strains were obtained by treatment with N-methyl-N′-nitro-N-nitrosoguanidine (Adelberg *et al.* 1965).

Culture media: Brain Heart Infusion (Difco) or Penassay Broth (Difco) was used

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as a broth. Minimal agar medium described by Sanderson and Demerec (1965) was used as a selective medium and essential amino acids at a concentration of 50μg/ml and/or streptomycin at a concentration of 1 mg/ml were added when needed.

Serological analysis of recombinants: O antigen was determined by slide agglutination test with appropriate O antiserum. Factor antiserum against O antigen 1, 4, 5, 9, 22, 23, 40 or 43 was prepared by the procedure described by Edwards and Ewing (1969). An unabsorbed serum against *S. paratyphi* A (2, 12) was used as an anti-12 reagent. Anti-Forssman F_A serum and anti-A serum were prepared by immunization of rabbits with *S. paratyphi* B 8006 (4, 5, 12) and with *S. riogrande* (40), respectively. Anti-*S. poona* and *anti-S. milwaukee* chicken immune sera were used as anti-H and anti-B sera.

Technique of genetic crosses: Genetic crosses were performed in the manner described by Jacob and Wollman (1961).

RESULTS

1. *Reciprocal crosses between* Salmonella *groups B and G*

The group B donor Hfr strain SW 1391 (1, 4, 5, 12) was crossed with a group G recipient F^- strain SK-po 01^r (13, 22) to yield *his*$^+$ *aro*$^+$ recombinants. When such recombinants were tested for agglutination with anti-1, anti-5, anti-4, 12 and anti-13, 22 sera, four different agglutination patterns were detected among them (Table 1): (i) donor-

Table 1. O antigen analysis of *his*$^+$ *aro*$^+$ recombinants obtained from the cross between *Salmonella abony* (group B: 1, 4, 5, 12) Hfr *his*$^+$ *aro*$^-$ donor and *S. poona* (group G: 13, 22), *S. riogrande* (group R: 40) or *S. milwaukee* (group U: 43) F^- *his*$^-$ *aro*$^+$ recipient

Recipient	O antigens and number of recombinants					Rough	Total
	4, 12	4, 5, 12	13, 22	40	43		
SK-po 01^r(13, 22)	174	94	25			19	312
SK-rio 01^r(40)	85	16		224		5	330
SK-mil 01^r(43)	67	0			813	0	880

like antigens but lacking factor 1 (4, 5, 12), 30.1% (94/312) of *his*$^+$ *aro*$^+$ recombinants; (ii) donor-like antigens but lacking factor 1 and 5 (4, 12), 55.7% (174/312); (iii) recipient-like antigens (13, 22), 8.0% (25/312); (iv) rough type, 6.2% (19/312). All the recombinants to which were transferred the O antigen 5 from the donor came to have the blood group A-active Forssman antigen F_A. The O antigens 4, 12 are determined at one locus closely linked to *his* and they replace O antigens 13, 22; to decide whether both of these factors are also determined at the same locus or only suppressed in the 4, 12 recombinants, the reverse cross is needed. The reverse cross was made, crossing a group G F′ donor SK-gr F′ (13, 23) to a group B recipient SK-83 (1, 4, 12). The donor selected marker was *his*$^+$ in this case. 47.6% (79/166) of the *his*$^+$ *str-r* recombinants received the O antigens 13, 23 and concurrently the blood group H-active antigen of

the donor (Table 2). The loss of the O antigens 1, 4, 12 occurred in all hybrids that received the O antigens 13, 23 determinants, thus confirming the probable allelism of the genetic determinants of antigens 4, 12 and 13, 22 (or 13, 23).

2. *Reciprocal crosses between* Salmonella *groups B and R*

The group B donor Hfr strain SW 1391 (1, 4, 5, 12) was crossed with a group R recipient SK-rio 01^r (40). Among those recombinants that had received the donor and the recipient *aro*$^+$ genes, 30.6% (101/330) of recombinants show the agglutination patterns of donor type antigens with anti-4, 12 or anti-5, and failed to agglutinate with anti-40 serum. Upon closer examination, four different agglutination patterns were detected among the *his*$^+$ *aro*$^+$ recombinants (Table 1): (i) donor-like antigens but lacking factor 1 (4, 5, 12), 4.9% (16/330) of *his*$^+$ *aro*$^+$ recombinants; (ii) donor-like antigens but lacking factor 1 and 5 (4, 12), 25.7% (85/330) *his*$^+$ *aro*$^+$; (iii) recipient-like antigen (40), 67.9% (224/330); (iv) rough type, 1.5% (5/330). In the reversed cross between a group R donor SK-rio F′ (40) and a group B recipient SK-83 (1, 4, 12), selecting the recombinants by the donor *his*$^+$ and the recipient *str-r* markers, 7.1% (31/433) of recombinants had the donor type antigen 40 and blood group A-active antigen (Table 2).

Table 2. O antigen analysis of *his*$^+$ *str-r* recombinants obtained from the cross between *Salmonella grumpensis* (Group G: 13, 23), *S. riogrande* (Group R: 40) or *S. milwaukee* (Group U: 43) F′ *his*$^+$ *str-s* donor and *S. paratyphi* B var. *odense* (Group B: 1, 4, 12) F$^-$ *his*$^-$ *str-r* recipient

Donor	O antigens and number of recombinants				Rough	Total
	13, 23	40	43	1, 4, 12		
SK-gr F′(13, 23)	79			87	0	166
SK-rio F′(40)		31		402	0	433
SK-mil F′(43)			45	896	5	946

3. *Reciprocal crosses between* Salmonella *groups B and U*

The group B donor Hfr strain SW 1391 (1, 4, 5, 12) was crossed with a group U recipient SK-mil 01^r (43). Among those recombinants that had inherited the donor *his*$^+$ and the recipient *aro*$^+$ genes, 7.6% (67/880) had the donor type antigens 4, 12 and lost the recipient type antigen 43; the rest had recipient-like O antigen 43 (Table 1). When the reversed cross was performed, crossing a group U donor SK-mil F′ (43) to a group B *his*$^-$ recipient SK-83 (1, 4, 12), three different antigen patterns were detected among the *his*$^+$ *str-r* recombinants (Table 2): (i) donor-like 43 and blood group-active antigen B, 4.7% (45/946) of *his*$^+$ *str-r* recombinants; (ii) recipient-like antigens 1, 4, 12, 94.8% (896/946); (iii) rough type, 0.5% (5/946).

4. *Adsorption of P22* (iota) *by recombinants*

Any of the *his*$^+$ *aro*$^+$ recombinants between the group B donor (1, 4, 5, 12) and the group G (13, 22), R (40) or U (43) recipient, which had inherited the donor O antigen 12, could not adsorb phage P22 (*iota*). This considered to indicate that no complete donor O antigen 12, which is receptor of antigen 1-converting phage P22, may be produced.

5. *Reciprocal crosses between* Salmonella *group G and R*

The *Salmonella* group G donor F′ strain SK-gr F′ (13, 23) was crossed with a *Salmonella* group R recipient F⁻ strain SK-rio 01^r (40). In a bacterial cross when the selected markers were *his*$^+$ of the donor and *str-r* of the recipient, 86.4% (38/44) of the recombinants had O antigens 13, 23 and the blood group H-active antigen of the donor type, and coincidently lost the O antigen 40 and the blood group A-active antigen of the recipient type (Table 3). The reversed cross was performed, crossing a *Salmonella* group R donor F′ strain SK-rio F′ (40) with a *Salmonella* group G recipient F⁻ strain SK-po 01^r (13, 22). The selected marker was donor *his*$^+$; 88.3% (357/405) of the *his*$^+$ *str-r* recombinants received the O antigen 40 and the blood group A-active antigen of the donor (Table 4). The anticipated loss of the O antigens 13, 22 and the blood group H-active antigen occurred in all hybrids that received the O antigen 40 determinant, thus confirming the probable allelism of the genetic determinants of O antigens 13, 22 (13, 23) and 40.

Table 3. O antigen analysis of *his*$^+$ *str-r* recombinants obtained from the cross between *Salmonella grumpensis* (group G: 13, 23) F′ *his*$^+$ *str-s* donor and *S. riogrande* (group R: 40) or *S. milwaukee* (group U: 43) F⁻ *his*⁻ *str-r* recipient

Donor	Recipient	O antigens and number of recombinants			Total
		13, 23	40	43	
SK-gr F′(13, 23)	SK-rio 01^r(40)	38	6		44
	SK-mil 01^r(43)	120		151	271

Table 4. O antigen analysis of *his*$^+$ *str-r* recombinants obtained from the cross between *Salmonella riogrande* (group R: 40) F′ *his*$^+$ *str-s* donor and *S. poona* (group G: 13, 22) or *S. milwaukee* (group U: 43) F⁻ *his*⁻ *str-r* recipient

Donor	Recipient	O antigens and number of recombinants			Rough	Total
		40	13, 22	43		
SK-rio F′(40)	SK-po 01^r(13, 22)	357	45		3	405
	SK-mil 01^r(43)	273		62	2	337

6. *Reciprocal crosses between* Salmonella *groups G and U*

The group G donor F′ strain SK-gr F′ (13, 23) was crossed with a group U recipient F⁻ strain SK-mil 01^r (43). Recombinants with *his*$^+$ of the donor and *str-r* of the recipient were selected. Among those recombinants that had received the donor *his*$^+$ gene, 44.3% (120/271) showed the donor type antigens 13, 23 and lost the recipient type antigen 43; the rest had the O antigen 43 like the recipient (Table 3). In the reversed cross between group U donor SK-mil F′ (43) and a group G *his*⁻ recipient SK-po 01^r (13, 22), 99.2% (516/520) of *his*$^+$ *str-r* recombinants showed the donor type antigen 43 and concurrently blood group B-active antigen, and lost the recipient type antigens 13, 22 (Table 5). All of the *his*$^+$ *str-r* recombinants to which were transferred O antigen 43 and the blood group B-active antigen had completely lost the H-activity to inhibit agglutination of O red cells by anti-H agglutinin in anti-*S. poona* chicken serum.

Table 5. O antigen analysis of *his*$^+$ *str-r* recombinants obtained from the cross between *Salmonella milwaukee* (group U: 43) F′ *his*$^+$*str-s* donor and *S. poona* (group G: 13, 22) or *S. riogrande* (group R: 40) F$^-$ *his*$^-$*str-r* recipient.

Donor	Recipient	O antigens and number of recombinants			Total
		43	13, 22	40	
SK-mil F′(43)	SK-po 01^r(13, 22)	516	4		520
	SK-rio 01^r(40)	333		19	352

7. *Reciprocal crosses between* Salmonella *groups R and U*

The group R donor F′ strain SK-rio F′ (40) was crossed with the group U recipient SK-mil 01^r (43). Among those *his*$^+$ *str-r* recombinants, 81.3% (273/337) had the O antigen 40 of donor type and lost the O antigen 43 of recipient type, two other antigenic patterns were detected among them (Table 4): O antigen 43 of recipient type, 18.1% (62/337) of *his*$^+$ *str-r* recombinants; rough type, 0.6% (2/337) of *his*$^+$ *str-r*. When the reverse cross was made, crossing the group U donor SK-mil F′ (43) with the group R *his*$^-$ recipient SK-rio 01^r (40), 94.6% (333/352) of the *his*$^+$ *str-r* recombinants received the O antigen 43 and blood group B-active antigen (Table 5).

8. *Manifestation mechanism of O antigen 1*

As described in experiments 1, 2 and 3, each F′·lac strain of group G (13, 23), R (40) and U (43) was crossed with an F$^-$ strain of group B (1, 4, 12). When recombinants with the *his*$^+$ gene from each donor were selected, it was found that some of them expressed the O antigenes 13, 23; 40 and 43 of the donor strains instead of the O antigens 1, 4, 12 of the recipient, respectively; and all of the recombinants that had donor type antigen carried phage P22 (*iota*), which was capable of antigenic conversion. This finding suggests that the genetic determinant of O antigen 1 was present in these recombinants; however, no O antigen 1 could be detected from them. *His*$^-$ mutants were obtained from these recombinants. Crosses between group D donor S-57 F′ (9, 12) and *his*$^-$ recipients derived from *his*$^+$ recombinants described above were performed in broth added anti-P22 (*iota*) serum to prevent antigenic conversion of O antigen 1 of the donor and transduction of *his*$^+$ from the donor by free P22 (*iota*). Among the *his*$^+$ *str-r* recombinants, a majority had the antigen 1 and donor-like antigens 9, 12 and lost the recipient antigens 40, 43 or 13, 23. It was considered that the donor *S. typhi* (S-57 F′) was not responsible for production of such recombinants with antigen 1 through conversion by P22 (*iota*), because the flagellar antigens of recombinants were b:1, 2 of the recipient *S. paratyphi* B var. *odense*, and because P22 was easily induced from the recombinants though it is generally difficult to induce P22 from *S. typhi* (S-57 F′) infected with P22 (*iota*). Also *his*$^+$ *str-r* recombinants with O antigens 1, 4, 5, 12 or 1, 4, 12 were produced when a group B strain S-3 F′ (4, 5, 12) was used as a donor.

DISCUSSION

Results of the present study indicate that the genetic determinants of O antigens

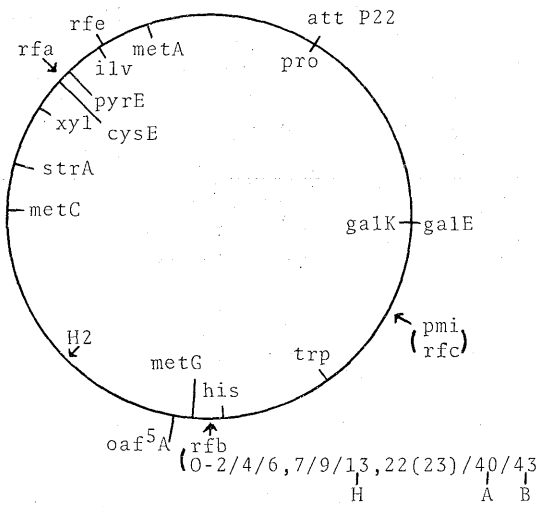

Fig. 1. Linkage map of *Salmonella*. Distances shown are taken from Sanderson (1970), Mäkelä and Stocker (1969). Gene symbols: *att P22*-prophage attachment site for P22; *cys*—cystine biosynthesis; gal-galactose metabolism *gal E* for UDP-galactose epimerase, *gal K* for galactose kinase; *H2*-phase 2 flagellar protein; *his*-histidine biosynthesis; *ilv*-isoleucine and valine biosynthesis; *met*-methionine biosynthesis; *oaf*^{5} *A*-O antigen 5 biosynthesis; *pmi*-phoshomannoisomerase; *pro*-proline biosynthesis; *pyr E*-pyrimidine biosynthesis; *rf*.-LPS biosynthesis (*rfa* of core; *rfb* and *rfe* of O-repeating unit; rfc, polymerization of repeating unit; *str A*-streptomycin; *trp*-tryptophan biosynthesis; *xyl*-xylose fermentation.

4, 12 of *Salmonella* group B, 13, 22 (or 13, 23) of group G, 40 of group R, and 43 of group U are situated on the *O* locus near *his* and probably allelic in crosses amongst groups B, G, R, and U (Fig. 1). This locus is now termed *rfb* (Nikaido *et al.* 1967). The position of the *rfb* cluster on the bacterial chromosome is well defined, lying between the *his* and *met G* loci, which are separated by 2 minutes transfer time or 2/138 of the whole chromosome (Stocker *et al.* 1966; Sanderson 1970). The cluster is thought to contain some structural genes for the enzymes transferring the some monosaccharides of the repeating unit into position (Nikaido *et al.* 1967; Mäkelä and Stocker 1969). This locus is presumably complex, containing genes for the synthesis of hexose nucleotides as well as genes for the transferases needed for putting together the specific part of the different lipopolysaccharide side chains (Stocker *et al.* 1966; Mäkelä and Stocker 1969). More systematic studies were made by analyzing the recombinants produced by crosses between group B *Salmonella* and group C_1 *Salmonella* (Nikaido *et al.* 1966). The results of genetic crosses showed that O antigen 13 and blood group H-active antigen, O antigen 40 and A-active antigen, O antigen 43 and B-active antigen, always coexisted respectively. This finding suggests that immunochemical similarities exist between *Salmonella* O antigens and human blood group-active antigens. So far, it has not been possible to separate the factor 13 from 22 or 13 from 23. Therefore, the corresponding alleles should perhaps be called *O-13, 22* and *O-13, 23*. It has

been shown that blood group H-active *S. poona* (13, 22) contains a terminal L-fucose (Yamamoto *et al.* 1963; Simmons 1965), B-active *S. milwaukee* a terminal α-D-galactose (Yamamoto *et al.* 1963; Lüderitz *et al.* 1965), while an α-*N*-acetyl-D-galactosamine is responsible for the A activity of *S. riogrande* (Yamamoto *et al.* 1963; Furukawa *et al.* 1972). The genes determining the O specificity of several other O antigen groups (A (Johnson 1967), C (Naide *et al.* 1965) and D (Mäkelä 1965; Johnson *et al.* 1965)) have been shown to map in the *O* locus. In *Escherichia coli* also a locus or gene cluster near *his* is the genetic determinant of *O* specificity (Ørskov and Ørskov 1962).

The genetic determinant of O antigen 5 can express its action only in an organism in which O antigen 4 is also manifested (Mäkelä 1965; Johnson *et al.* 1965). In the cross between group B (1, 4, 5, 12) donor and group U (43) recipient when the donor *his*$^+$ marker was selected, none of the *his*$^+$ recombinants with O antigens 4, 12 had the O antigen 5 and Forssman antigen F_A. Thus it was found to be responsible for incomplete genetic or chemical structure of O antigen 4 transferred from group B donor *S. abony*. In these crosses it was not possible to separate O antigen 5 and Forssman F_A-active antigen, O antigen 13 and blood group H-active antigen, O antigen 40 and A-active antigen, and O antigen 43 and B-active antigen, respectively from each other; these findings suggest that serological intimate relations exist between *Salmonella* O antigens and blood group-active antigens through their similar oligosaccharide structures of antigenic determinants.

Salmonella O antigen 12 found in *Salmonella* groups A, B and D is thought to be the receptor of phage P22 (*iota*) (Iseki and Kashiwagi 1957). However, *his*$^+$ recombinants with O antigen 12 which were obtained from the cross between group B (1, 4, 5, 12) donor and group G (13, 22), R (40) or U (43) recipient could not adsorb P22 (*iota*). Similar recombinants were obtained in a cross between a group B Hfr strain (4, 5, 12) and a group C_1 F^- strain (6, 7) (Naide *et al.* 1965). The finding noted above suggests that the structure of O antigen 12 of such recombinants is defective in adsorption ability of P22 as compared with that of natural O antigen 12 in *Salmonella* groups A, B and D.

Any of the *his*$^+$ recombinants which accepted O antigen 40, 43 or 13, 23 and produced P22 (*iota*) were not capable of expressing O antigen 1. O antigen 1 in hybrids which received initially O antigen 40, 43 or 13, 23 was produced after introduction of *his*$^+$ and O antigen 12 markers into them by conjugation with F′ strains. This finding, coupled with the fact that the O antigen 1 is not found in *Salmonella* groups A, B and D bacteria without the O antigen 12, suggests that the genetic determinant of O antigen 1 can express its action only in an organism in which O antigen 12 is manifested. It was reported that the formation of O antigen 15 is a prerequisite for the formation of O antigen 34 in *Salmonella* group E (Uetake and Hagiwara 1960). There is a close resemblance between the two results mentioned above.

SUMMARY

From the genetic studies, it has become clear that the specific structures of the O antigens of 13, 22 or 13, 23 (group G) with blood group H activity, 40 (group R) with

A, 43 (group U) with B and 4, 12 (group B), that is, the structures of the S-specific side chains, are determined at a gene locus (the *O* locus) close to the *his* locus in *Salmonella*.

Phage P22 (*iota*) can produce the O antigen 1 only in an organism in which the O antigen 12 is manifested.

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