The Sensitivity of Smooth and Rough Gram-Negative Bacteria to the Immune Bactericidal Reaction (34472)

LOUIS H. MUSCHEL AND LINDA J. LARSEN

Department of Microbiology, University of Minnesota Medical School, Minneapolis, Minnesota 55455

S-R variation of gram-negative organisms, particularly members of the colon typhoid group, signifies a change from a smooth to rough form, resulting in the loss of the O antigen (1). Generally, an R variant becomes rough morphologically although the serological and morphological changes may occur independently (2). In contrast to S forms, R variants are generally more susceptible to the bactericidal action of normal serum mediated by the complement (C) system. Although this finding has been well known for many years (3) and repeatedly confirmed (4), it has never been adequately explained. An obvious possible reason for these results is that the substance in the outer layer of S forms that functions in the control of permeability and that may be attacked by C (5) is more resistant to the C enzymes. At this time, it would be difficult or impossible to test this postulate in the absence of definitive information relating to the enzyme- substrate complex that is involved in cell death by the C system. Another possibility would involve the accessibility or distance of the C "substrate" from the locus of the antigen–antibody complex that activates C (6). It is not unlikely that the immunodeterminant groups of the S form may be more distant than the core region (2) from the C substrate. In addition, it is conceivable that C may be inactivated by determinants that are C-enzyme inhibitors.

These considerations all imply that there is an inherent difference in the sensitivity of S and R forms to the C system. Since the immune bactericidal reaction requires the presence of antibody (7), conceivably normal antibody may be relatively lacking against the specific antigenic determinants of the S strains but present in higher concentration against R strains. Only a limited number of distinct R mutants exist (2) so that antibody formation may be greater against the antigenic determinants of the R forms simply because of greater antigenic stimulation or for other unknown reasons. Experiments were performed, therefore, to determine whether the relative resistance of certain S forms might be related to the lack of bactericidal antibody against these forms in normal serum.

Materials and Methods. Serum bactericidal activity was determined by a quantitative photometric assay modified for the determination of whole serum activity (8) or of C with bacteria optimally sensitized with antiserum (9). The organisms were obtained from different sources: *Escherichia coli* 0111-B_4 and *E. coli* J-5 from Dr. E. C. Heath, The Johns Hopkins School of Medicine. The J-5 strain is a mutant of *E. coli* 0111-B_4. The mutant produces an incomplete polysaccharide lacking colitose, the terminally located dideoxyhexose of the parent strain, and other sugars distal to galactose in the complete polysaccharide (10). *Vibrio cholerae* type Inaba 20-A-10, *Salmonella typhi* 0901, *S. typhi* Ty 2, *S. typhi* Mrs. S., and *Paracolobactrum ballerup* were all obtained from Mr. A. Abrams, WRAIR, Washington, D. C. These *S. typhi* strains and *P. ballerup* have been widely used in studies of typhoid fever immunology (11). *V. cholerae* CA-385, a rough strain, was obtained from Dr. R. Finkelstein of the University of Texas Southwestern Medical School, Dallas. *Salmonella anatum* and *Salmonella newington* were obtained from Dr. W. Ewing, National Communicable Disease Center, Atlanta, Ga.

TABLE I. Bactericidal Titration of Normal Guinea Pig Serum Itself and of Its C Activity in the Presence of Antiserum Against *E. coli* Strains 0111-B_4 and J-5.

	Titers[a]	
	J-5	0111-B_4
Guinea pig serum alone	15	2.9
Guinea pig serum with a constant amount (1×10^{-3} ml) of antiserum against 0111-B_4	67	45
Guinea pig serum with a constant amount (1.6×10^{-3} ml) of antiserum against J-5	42	20

[a] Represent the reciprocal of the serum amount needed for a 50% bactericidal end point. The titers against J-5 and 0111-B_4 are significantly different ($p < .05$) since the standard deviation of relative serum titers in the photometric assay is only about 10% of these titers; see Ref. (8).

S. anatum with the O-factors, 3, 10 is converted to *S. newington* by infection with phage *epsilon*-15 resulting in the replacement of factor 10 by factor 15.

Results. Reactions with E. coli *0111-B and J-5.* Normal guinea pig serum was titrated against *E. coli* strains 0111-B and J-5. In addition, this sample of serum was titrated for C activity in the presence of an excess of antiserum against the homologous organisms. The results (Table I) indicated that the S strain, 0111-B_4 required about five times as much normal guinea pig serum for a 50% end point as the R mutant, J-5. When homologous antiserum was used for sensitization, C titers against the two strains were quite similar with titers of 45 against 0111-B_4 and 42 against J-5 although the 0111-B_4 antiserum provided a more effective sensitization against J-5.

Reactions with V. cholerae. A similar experiment was performed with a morphologically smooth and rough strain of *V. cholerae.* The results (Table II) demonstrate even more dramatically that the relative resistance of smooth strain 20-A-10 to normal serum is a consequence of the lack of antibody against that organism in normal serum. Strain 20-A-10 was 10 times more resistant to normal guinea pig serum than CA-385. In the presence of a rabbit antiserum against 20-A-10, however, the sensitivity of 20-A-10 was almost 30 times greater than in its absence. The same antiserum had no apparent effect in the titration of C against CA-385 possibly because of a lack of serological cross-reactivity between the two strains.

Reactions with S. newington *and* S. anatum. Normal guinea pig serum titers against *S. newington* and *S. anatum* were 35 and 18, respectively, although the latter organism is relatively rougher as determined by growth in broth. When each organism was sensitized with optimal amounts of an homologous antiserum, *S. newington* was still more sensitive to guinea pig serum as a C source with a titer of 59 compared with a C titer of 45 against *S. anatum.*

Reactions with S. typhi. Because *S. typhi* possesses at least two distinct somatic antigens, O and Vi (11), it was of interest to compare C titers against a strain of that organism, Ty2, which possesses both those antigens and against strain 0901 which lacks the Vi antigen. Sensitization was accomplished by anti-O (antiserum prepared against strain 0901), anti-Vi (antiserum prepared against *P. ballerup*) and an antiserum containing both anti-O and anti-Vi (anti-Ty2). It is well known that strain Ty2 is more resistant to normal serum than strain 0901 (8), and verified by the results in this study (Table III). Moreover, irrespective of the source of antibody, strain Ty2 was more resistant to C than strain 0901. The relative resistance of strain Ty2 to normal serum could not be attributed, therefore, to a lack of anti-Vi in such serum. In fact, the C titer was lower with anti-Vi than with anti-O. Finally, strain Mrs. S., a rough strain, was no

TABLE II. Bactericidal Titration of Normal Guinea Pig Serum Alone and in the Presence of Antiserum Against *V. cholerae* Strains CA-385 and 20-A-10.

	Titers		Titer vs. CA-385 /titer vs. 20-A-10
	CA-385	20-A-10	
Guinea pig serum alone	36	3.5	10.3
Guinea pig serum with constant amount of antiserum (1×10^{-4} ml) against 20-A-10	37	100	0.37

more resistant than Ty2 to normal guinea pig serum and even more resistant than 0901.

Discussion. A great amount of work has indicated that morphologically and serologically rough strains are more sensitive than smooth strains to the bactericidal action of normal serum. In this study for example, the CA-385 rough strain of *V. cholerae* was considerably more sensitive than smooth strain, 20-A-10. Exceptions to this generalization, however, were noted: the relatively greater resistance of the rough organism, *S. typhi* Mrs. S., compared with the smooth 0901 strain of *S. typhi*; the greater sensitivity of *S. newington* compared with *S. anatum.*

In accord with most observations indicating greater sensitivity of rough forms, the R variant, *E. coli* J-5, was about five times more sensitive to normal guinea pig serum than the S-form, *E. coli* 0111-B_4. Yet in the presence of 0111-B_4 antiserum, there was only a 1.5-fold difference in C titer. Thus, the marked difference in resistance of the two organisms to normal serum may be attributed to a lack of adequate antibody against 0111-B_4 in normal serum and not to an inherent resistance to C. Since the core polysaccharide is present in relatively large numbers of organisms compared to the limited distribution of the specific determinants in strains similar to 0111-B_4 (2), the results strongly suggest that antibody to the O-specific region of *E. coli* and other smooth organisms may often be relatively lacking in normal sera. There are, however, gram-negative organisms such as strains of *P. ballerup, Salmonella paratyphi C.,* and *Salmonella typhimurium* which, under the usual experimental conditions are completely refractory to C even with adequate sensitization by specific antiserum (12). These are the organisms to be considered in studies relating to C resistance.

TABLE III. Bactericidal Activity of Normal Guinea Pig Serum Against *S. typhi* 0901 and *S. typhi* Ty2 in the Presence and Absence of Sensitizing Antisera.

Antiserum	Titers		
	0901	Ty2	Mrs. S
Anti-0901 (anti-O)	106	56	ND*
Anti-*P. ballerup* (anti-Vi)	ND	28	ND
Anti-Ty2 (anti-O + anti-Vi)	76	56	ND
No antiserum	20	9	8

* ND = not done.

The role of the Vi antigen in conferring resistance of bacteria to normal serum (8) was extended in this work to resistance to C. *S. typhi* 0901 was more sensitive to C than strain Ty2 even in the presence of anti-Ty2. With strain Ty2 and also with *P. ballerup* loss of the Vi antigen is associated with increased C sensitivity (12). Possibly certain structures of the bacterial cell may prevent access of C to its "substrate." Similarly, gram-positive organisms are absolutely resistant to the C system probably because their cell walls prevent access of C from the site of its activation on the cell surface to the susceptible cell membrane. The protoplasts of gram-positive cells are therefore, C sensitive (13).

Summary. Rough strains of gram-negative bacteria are not necessarily more sensitive than smooth strains to the bactericidal action of normal serum or C. The resistance of certain smooth strains to C may merely reflect a lack of normal antibody in serum. Other smooth organisms may be refractory to C even when sensitized with homologous antiserum. In such instances, it is believed that

the C-sensitive substance may be too far removed or inaccessible to the C enzymes activated by antigen–antibody complexes on the cell surface (6).

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