MICROBIAL PATHOGENICITY

K1, K5 and O antigens of *Escherichia coli* in relation to serum killing *via* the classical and alternative complement pathways

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Summary. The sensitivity of *Escherichia coli* strains to 80 % normal human serum (NHS) and the relative importance of the classical and alternative complement pathways was assessed in relation to K1, K5, and O antigen carriage. Strains of each of the common O-serogroups, O1, O2, O4, O6, O7, O9, O18 and O75, smooth strains not typable (NT) with these antisera and auto-agglutinable (AA) strains were studied. Of the 166 strains studied, 37 carried the K1 antigen and 45 the K5 antigen. The variation in sensitivity to NHS between different O-serogroups reported previously was confirmed. Although carriage of the K1 and K5 antigens varied with O-serogroup, this did not explain the differences either between or within O-serogroups. Strains with the K1 or K5 antigen were significantly more resistant to the alternative complement pathway than strains without these antigens. However, this appeared to be more related to the O-serogroups with which they were associated; 37 of 50 O2, O4, O6 and AA strains were affected by complement through both pathways but 20 of 30 O7, O18 and O75 strains were affected by the classical pathway alone and 16 of 20 O9 and NT strains were affected by the alternative pathway alone.

Introduction

The sensitivity of *Escherichia coli* strains to the bactericidal activity of normal human serum (NHS) is related to their O-serogroup^{1,2} and there are indications that sensitivity to the classical and alternative complement pathways is similarly related to O antigen.^{3,4} However, within any particular O-serogroup strains vary widely in sensitivity. It has been suggested that the K1 antigen specifically blocks the alternative complement pathway.^{5–8} This antigen occurred commonly amongst isolates from urinary tract infection or bacteraemia in an earlier study and was also related to O-serogroup.⁹

The purpose of the present study was to determine if carriage of the K1 antigen explained the variation in sensitivity to serum either within or between O-serogroups. The K5 antigen occurs with a similar frequency to K1 and also varies with O-serogroup.⁹ Both antigens are poorly immunogenic in man because they mimic host molecules: K1 is a homopolymer of N-acetylneuraminic acid^{10,11} and K5 is identical to desulphoheparin.¹² Therefore, to compare K1-carrying strains with similar strains carrying a different, specific K antigen, K5-carrying strains were

also assessed. Strains carrying neither of these K antigens were also included to establish the general range of serum sensitivity in the serogroups studied.

Materials and methods

Bacteria

The methods of identification and O-serogrouping of the *E. coli* strains have been described previously.¹³ Our stock culture collection, all isolated from suprapubic aspirates of urine, contained 136 strains belonging to the eight common urinary O-serogroups (O1, O2, O4, O6, O7, O9, O18 and O75). A further ten smooth strains not typable (NT) with antisera to these O-groups and 10 strains that were auto-agglutinable (AA) in NaCl 0.85 % w/v were selected at random from the stock culture collection. Ten additional AA strains isolated from midstream specimens of urine and carrying K1 or K5 antigens were also included in the study. Strains carrying the K1 or K5 antigen were identified with specific bacteriophages^{9,14} and strains having neither antigen were designated KU (K-Unknown): the capsular status of these strains was not determined and they were simply regarded as representative of the range of strains not carrying K1 or K5 antigens.

Serum and reagents

Fresh blood was taken from one of a small panel of

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Figure. Examples of grades of response of *E. coli* in bactericidal assays. Grade 1, all counts < 100%, 1-h count < 10%; grade 2, all counts < 100%, 1-h count 10–99%; grade 3, 1-h count > 100%, 2- and 3-h counts < 100%; grade 4, 1- and 2-h counts > 100%, 3-h count < 100%; grade 5, all counts > 100% but falling at some stage; grade 6, progressive rise at each hourly interval.

donors on the day of each experiment and allowed to clot. After centrifugation, the serum was removed and passed through a 0.22- μ m membrane filter (type GS; Millipore).

For elimination of the classical complement pathway, a solution of 100 mM ethyleneglycoltetra-acetic acid (EGTA; Sigma), supplemented with 100 mM $MgCl_2$ (MgEGTA) in normal saline (NaCl, 0.9% w/v), was prepared as described by Fine *et al.*¹⁵ and sterilised by filtration through a 0.22 μ m membrane filter.

Assay of serum bactericidal activity

Details of the assay have been given previously.³ Briefly, 0.2 ml of a suspension containing *c.* 2×10^5 actively dividing bacteria in normal saline was added to 1.6 ml of fresh NHS plus 0.2 ml of normal saline (NHS) or MgEGTA (NEMg) giving a final serum concentration of 80% v/v. Heat-inactivated serum (56°C for 30 min) plus saline (HIS) or MgEGTA (HIEMg) were used as controls. Viable counts were made at the start and after incubation for 1, 2 and 3 h at 37°C. Results were calculated as the percentage of the initial viable count surviving at each hourly interval. For each strain, all tests were done three times on different days with serum from at least two different donors. The mean responses were graded from 1 (when the viable count was < 10% of the inoculum by the first hour), to 6 (when the count increased at each hourly interval). An example of each grade and its definition are given in the figure; the definitions are those used by Hughes *et al.*² but slightly modified, as a small number of strains could not be graded with their original definitions. Any strain showing a grade 6 response when first assessed was subsequently tested in

parallel with a serum-sensitive strain of the same O-serogroup to establish that the lack of killing was not due to a deficiency of complement or antibody. This was not considered necessary with other grades of response as some killing occurred and the grade of response was the same in the three tests with serum from at least two donors.

Distinction between classical and alternative complement pathways

The addition of MgEGTA to NHS blocks the classical pathway while allowing the alternative pathway to function. Therefore, any difference in killing between NHS and NEMg was attributed to the classical pathway whereas a difference between NEMg and HIEMg was attributed to the alternative pathway. When no killing occurred in NEMg, the bactericidal activity in NHS was considered to be due to the classical pathway alone. When there was no difference between NHS and NEMg, any bactericidal activity in NHS was regarded as due to the alternative pathway alone, simply for ease of expression, although it could be that the classical pathway alone, or both pathways in combination, exerted the same effect in NHS as the alternative pathway alone did in NEMg.

Statistical analyses

The significance of differences in grades of response between various groups of strains was assessed by the Wilcoxon sum of ranks (T) test with the probability being determined by Z transformation. The significances of differences in proportions was assessed by the χ^2 test with Yates's correction.

Results

The susceptibilities of all 166 *E. coli* strains to NHS are shown in table I in relation to the carriage of O and K antigens. The mean grades of response in the different serogroups were similar to those reported previously² although, with the exception of strains of serogroup O6, they were slightly more serum resistant. Strains of serogroup O18 were the least serum sensitive and were significantly less sensitive than strains of serogroups O1, O2, O4, O6, O75, NT and AA ($p < 0.05$ – > 0.002). Only five serogroups (AA, O4, O6, O9 and O18) had completely serum-resistant (grade 6) strains. In all serogroups, fully sensitive strains were found but comprised a minority, except among AA strains where 16 of 20 were fully sensitive; the other four AA strains all carried the K1 antigen and one was the only serum-resistant K1 strain found in this study.

K1, K5 and KU strains displayed a wide range of responses to serum but only one K1 strain was completely resistant to serum. On the other hand, with only 13 (35.1%) of 37 K1 and 10 (22.2%) of 45 K5 strains was the viable count reduced during the first hour of the assay (grades 1 and 2) in contrast to 39

Table I. Sensitivity of 166 strains of *E. coli* to normal human serum in relation to K1, K5 and O antigens

O-serogroup	Total	Number of strains (number with K1/K5)† responding with serum sensitivity grade*						Mean grade
		1	2	3	4	5	6	
O1	20 (14/0)	6 (3/0)	4 (3/0)	8 (8/0)	0	2	0	2.4
O2	11 (4/5)	2 (1/1)	2 (0/1)	3 (0/3)	1	3 (3/0)	0	3.1
O4	16 (0/1)	5	5	3 (0/1)	0	2	1	2.5
O6	22 (0/3)	3 (0/1)	4 (0/1)	6 (0/1)	5	1	3	3.3
O7	12 (8/0)	1 (1/0)	0	2 (1/0)	7 (6/0)	2	0	3.8
O9	13	2	1	4	1	2	3	3.7
O18	23 (4/16)	2	0	1 (1/0)	11 (2/9)	6 (1/5)	3 (0/2)	4.2
O75	19 (0/15)	1 (0/1)	0	12 (0/8)	5 (0/5)	1 (0/1)	0	3.3
NT	10	2	4	1	1	2	0	2.7
AA	20 (7/5)	16 (3/5)	2 (2/0)	0	0	1 (1/0)	1 (1/0)	1.0
Total	166 (37/45)	40 (8/8)	22 (5/2)	40 (10/13)	31 (8/14)	22 (5/6)	11 (1/2)	3.0

NT, not typable with antisera to the eight common O-serogroups.

AA, auto-agglutinable (agglutinates in all sera and in saline alone).

*Grade of response in bactericidal assays, see figure.

†Omitted when neither antigen was present.

Table II. Sensitivity of 110 strains of *E. coli* to the alternative complement pathway in relation to K1, K5 and O antigens

O-serogroup	Total	Number of strains (number with K1/K5)† responding with serum sensitivity grade*						Mean grade
		1	2	3	4	5	6	
O1	10 (7/0)	0	2 (1/0)	0	1 (1/0)	3 (2/0)	4 (3/0)	4.7
O2	10 (3/5)	0	1 (0/1)	1 (1/0)	1	4 (0/3)	3 (2/1)	4.7
O4	10 (0/1)	0	3	3	1	0	3 (0/1)	3.7
O6	10 (0/1)	0	2 (0/1)	3	1	2	2	3.9
O7	10 (7/0)	0	1 (1/0)	1	0	1 (1/0)	7 (5/0)	4.9
O9	10	2	0	7	0	0	1	2.9
O18	10 (3/5)	0	1	0	0	1	8 (3/5)	5.5
O75	10 (0/7)	0	0	1	1	2 (0/2)	6 (0/5)	5.3
NT	10	2	3	2	1	2	0	2.8
AA	20 (7/5)	1	11 (1/4)	1	3 (2/1)	1 (1/0)	3 (3/0)	3.0
Total	110 (27/24)	5	24 (3/6)	19 (1/0)	9 (3/1)	16 (4/5)	37 (16/12)	4.0

NT, not typable with antisera to the eight common O-serogroups.

AA, auto-agglutinable (agglutinates in all sera and in saline alone).

*Grade of response in bactericidal assays, see figure.

†Omitted when neither antigen was present.

(46.4%) of 84 KU strains. With respect to grade 1 and 2 responses, only the difference between K5 and KU was statistically significant ($\chi^2 = 6.297$; $p < 0.025$); there were no significant differences in mean grade of response (K1 3.0; K5 3.1; KU 3.0) and although K1 and K5 strains accounted for 82 (49.4%) of the 166 strains, only three of the 11 completely resistant (grade 6) strains carried these antigens.

A high rate of carriage of K1 or K5 in an O-serogroup was not associated with a high mean grade of response. Serogroups O1 and O7 both had a high proportion of K1 strains and no K5 strains but, whereas O7 had the highest mean grade apart from O18, O1 had the lowest mean grade of response apart from AA strains. In both O1 and O7 serogroups the most resistant strains (grade 5) were KU. Of the 19 strains of serogroup O75, 15 carried the K5 antigen and although the six strains with the highest grades of response (4 and 5) all had this antigen, so did the strain which was most sensitive (grade 1). Furthermore, the

mean grade of response of O75 strains was 3.3, the same as for the 22 strains of O6, of which only three carried K5. In the most serum-resistant serogroup (O18), a high proportion (20 of 23) of strains carried either the K1 or K5 antigen. A similar proportion (9 of 11) of O2 strains carried these antigens but this serogroup was much more sensitive to serum than O18. The three most resistant (grade 5) O2 strains carried the K1 antigen whereas seven of the nine most resistant (grades 5 and 6) O18 strains carried the K5 antigen and only one had K1. No strains in serogroup O9 carried either K1 or K5 antigens, but this group gave the second highest mean grade of response and contained the highest proportion of fully resistant (grade 6) strains. NT, and serogroups O1 and O4 had a similar low mean grade of response (2.7, 2.4 and 2.5 respectively) but no NT strain carried either K antigen, 14 of 20 O2 strains had K1 and only one of 16 O4 strains had K5.

The susceptibilities of 110 selected strains to the

Table III. Susceptibility of 110 strains of *E. coli* to the classical and alternative complement pathways in relation to K1, K5 and O antigens

O-serogroup	Total	Number of strains (number with K1/K5 antigens)* and complement pathways activated			
		Classical	Alternative	Both	Neither
O1	10 (7/0)	4 (3/0)	2 (1/0)	4 (3/0)	0
O2	10 (3/5)	3 (2/1)	0	7 (1/4)	0
O4	10 (0/1)	2 (0/1)	0	7	1
O6	10 (0/1)	1	1	7 (0/1)	1
O7	10 (7/0)	7 (5/0)	1	2 (2/0)	0
O9	10	0	8	1	1
O18	10 (3/5)	7 (3/4)	0	2	1 (0/1)
O75	10 (0/7)	6 (0/5)	1	3 (0/2)	0
NT	10	0	8	2	0
AA	20 (7/5)	2 (2/0)	1	16 (4/5)	1 (1/0)
Total	110 (27/24)	32 (15/11)	22 (1/0)	51 (10/12)	5 (1/1)

NT, not typable with antisera to the eight common O-serogroups.

AA, auto-agglutinable (agglutinates in all sera and in saline alone).

*Omitted when neither antigen was present.

alternative complement pathway are shown in relation to carriage of O and K antigens in table II. These strains included all the NT and AA strains together with 10 strains of each O-serogroup selected to include the most serum-sensitive strain, the most serum-resistant strain and eight others selected at random after excluding any other fully resistant (grade 6) strains. The selection of strains resulted in a small rise in mean grade of response to NHS in O1 (2.4–2.7) and O75 (3.3–3.4) strains, no change in O7 strains (3.8), a slight fall in O2 (3.1–2.9) and O4 (2.5–2.2) strains and a more marked decrease, due to the deliberate omission of two of the three fully resistant (grade 6) strains, in O6 (3.3–2.8), O9 (3.7–3.1) and O18 (4.2–3.4) strains. With the exception of the NT strains and those of serogroup O9, there was a marked increase in the mean grade of response to the alternative complement pathway compared to that with NHS. All groups apart from NT contained at least one fully resistant (grade 6) strain and only three groups (O9, NT and AA) contained promptly sensitive (grade 1) strains.

Again, K1 and K5 strains covered a wide range of susceptibilities but none of the five promptly sensitive strains (grade 1) carried either antigen. Only 11.1% (three of 27) of K1 strains and 25% (six of 24) of K5 strains showed a grade 1 or 2 response, compared with 33.9% (20 of 59) of KU strains; none of these differences was statistically significant. However, the mean grades of response for the K1 strains (5.1), and for the K5 strains (4.7), were significantly greater than for the KU strains (3.0; $p < 0.001$ and $p < 0.01$, respectively). These differences were largely due to the excess of K1 and K5 strains among those which were fully resistant (grade 6).

When the mean sensitivities of individual serogroups to complement activated *via* the alternative pathway were considered, serogroup O18 was again the most resistant and all of the eight O18 strains carrying K1 or K5 antigens were fully resistant. In the next most resistant group (O75), five of the six fully

resistant strains carried the K5 antigen. In other serogroups, K1 and K5 strains were found among both the most sensitive and the most resistant representatives. Serogroups O1 and O7 both included seven K1 strains, gave similar mean grades of response and showed a preponderance of K1 strains among those that were fully resistant. However, the most sensitive strain in each of these two groups also carried K1 antigen. Serogroup O2, which included three K1 and five K5 strains, had a similar mean grade of response to serogroups O1 and O7. Again, although the three fully resistant strains carried either K1 or K5 antigens, so did the two most sensitive strains. Serogroups O4 and O6 each contained one strain with the K5 antigen, but whereas this was one of the fully resistant strains in serogroup O4, it was one of the most sensitive in serogroup O6. Among the AA strains, all three fully resistant strains carried the K1 antigen and indeed six of the seven most resistant (grades 4, 5 and 6) carried K1 whereas only one of the 13 most sensitive (grades 1, 2 and 3) carried this antigen.

The responses of the 110 selected strains to the two complement pathways are summarised in relation to O and K antigens in table III. It is clear that sensitivity to the pathways is related to O-serogroup. Whereas 21 of the 30 O2, O4 and O6 strains and 16 of the 20 AA strains were affected by both pathways, 20 of the 30 O7, O18 and O75 strains were only sensitive to the classical pathway and 16 of the 20 O9 and NT strains appeared to be affected by the alternative pathway alone. Among the strains carrying K1 or K5 antigens, the response again appeared to be related to O-serogroup: none of the eight O18 strains and only four of the 14 O7 and O75 strains were sensitive to the alternative pathway, whereas nine of the 16 O1 and O2 strains were sensitive to complement *via* this pathway.

Discussion

This study provides further evidence that the sen-

sitivity of *E. coli* to normal human serum is related to the O-serogroup^{1,2} and has confirmed our earlier impression^{3,4} that not all strains of *E. coli* are sensitive to complement killing by both the classical and alternative pathways. It is clear that the response to the two pathways of complement activation is at least partly related to O-serogroup; most O2, O4, O6 and AA strains (37 of 50) were affected by both pathways, but 21 of 30 O7, O18 and O75 strains were affected by the classical pathway alone or by neither pathway, and 17 of 20 O9 and NT strains showed no change in sensitivity to serum when the classical pathway was blocked. Given the association of the K1 and K5 antigens with particular O-serogroups,⁹ it is likely to be misleading to study K antigens without reference to O-group. Moreover, particular O:K combinations have been considered more virulent than others.^{16,17}

Reports have indicated that a high proportion of *E. coli* K1 strains are resistant to serum^{18,19} but only one of 37 such strains in this study was fully resistant to NHS 80% for 3 h (grade 6). However, 24 of them grew for at least the first hour of the test (grades 3, 4, 5 and 6). Similarly, only two of 45 K5 strains were fully resistant to serum but 35 grew for at least the first hour of the test. It is our experience that most of the strains which grow for 1 h in 80% serum will grow for 3 h, and be regarded as resistant, if the serum concentration is reduced to 40% as in some other assays.¹⁸ Furthermore, the mean grades of response for K1, K5 and KU strains did not differ significantly. Such findings emphasise the importance of the criteria for a valid test of serum bactericidal activity put forward by Taylor.²⁰

In our experience, complete resistance to normal human serum is infrequent (table I) and largely confined to serogroups O6, O9 and O18. Of these three groups, only O18 contains K1 strains and, although an occasional O6:K5 is found, the K5 antigen is only common among O18 strains. Serogroups O1 and O7 contained a similar high proportion of K1 strains but O1 was the most serum-sensitive group apart from AA, whereas O7 was the most serum-resistant apart from O18. Pluschke and Achtman²¹ found that almost all K1 *E. coli* strains belonging to serogroups O7 and O18 were resistant to non-immune rat serum whereas O1:K1 strains were sensitive. However, when specific anti-lipopolysaccharide antibodies were added, the O7 and O18 strains were also killed. It is well known that NHS contains antibodies to the common *E. coli* O-serogroups, so classical pathway activity would not have been impaired in the present study. Such considerations show that differences in sensitivity to NHS between the O-serogroups is not explained by differences in the carriage of the K1 or K5 antigen.

Only four of the AA strains were not killed very rapidly by NHS; all of these four strains carried K1 and one of them was the only K1 *E. coli* in this collection which was fully serum-resistant. Serum-resistant AA:K1 strains have been reported pre-

viously^{18,22} and this may suggest that in AA strains K1, but not K5, can protect against complement killing. However, Allen *et al.*²³ introduced a K1 plasmid into a non-K1 rough strain of *E. coli* and observed only a slight decrease in serum susceptibility which was significant in 5% but not 10% serum. The relatively reduced serum susceptibility seen in some but not all of our AA:K1 strains may be a reflection of the O-serogroups from which they arose. Apart from these AA strains there was no suggestion that carriage of either K1 or K5 antigen accounted for the variation in serum sensitivity within O-serogroups.

It has been suggested that because the K1 antigen is a homopolymer of sialic acid it should block alternative pathway activity.⁵⁻⁸ Although many of the K1 strains did become fully resistant to serum when the classical pathway was blocked, this was not always the case and one O1:K1 strain appeared to be affected only by the alternative pathway. The differences in the response of these strains to serum could indicate that blocking of the alternative pathway is related to the amount of K1 on the surface of the strains and this is currently being investigated. Many of the K5 strains were similarly resistant to the alternative pathway and both K1 and K5 strains gave significantly greater mean grades of response than the KU strains. Furthermore, apart from the AA strains, the more sensitive serogroups (mean grade ≤ 3.9) were those with one or no strains carrying either K1 or K5 antigen, whereas those that were more resistant (mean grade ≥ 4.7) had seven or eight strains with these antigens. However, only in serogroup O18 were all K1 and K5 strains fully resistant and in several serogroups such strains were among the most sensitive isolates.

The K5 antigen, like K1, is considered to be an important virulence factor of *E. coli* and the two antigens are indeed similar in many respects: they share common steps in their biosynthesis, both are relatively non-immunogenic because they mimic host molecules and both are common among isolates from patients with urinary tract infection or bacteraemia. However, the sensitivity of K5 strains to human serum has received little attention. This study has shown that K5 strains are also similar to K1 strains in that most show a delayed sensitivity to NHS and are resistant to the alternative complement pathway. In spite of these similarities, the strong association between K1 strains and neonatal meningitis^{24,25} is not seen with K5 strains. Therefore, it is unlikely that resistance to the alternative pathway accounts for the very high incidence of *E. coli* K1 strains in neonatal meningitis. It may be that immunological tolerance, resulting from the structural similarity of K1 polysaccharide and embryonic neural cell adhesion molecules,²⁶⁻²⁸ is more important.

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