Sex Pili as Immunogens

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SUMMARY

Groups of chickens were vaccinated intravenously with live cultures of *Escherichia coli* K12 possessing transfer factors F, I, A2 or no transfer factor (Tra⁻) and their immunity challenged by injecting them intravenously with an F⁺ strain of *Salmonella heidelberg* or an F⁺ or I⁺ strain of *Salmonella typhimurium*. Compared with the Tra⁻ strain, vaccination with the F⁺, but not the I⁺ or A2⁺, K12 strain significantly reduced the mortality rate caused by the F⁺ salmonella strains; vaccination with the I⁺ or A2⁺, but not the F⁺, K12 strain significantly reduced the mortality rate caused by the I⁺ salmonella strain.

INTRODUCTION

Following the initial observation of Ørskov & Ørskov (1960) that agglutinating antibodies can be produced against the sex pili determined by transfer factor F, sex pilus antibody has been demonstrated by other techniques. These include direct observation by electron microscopy of the attachment of antibody molecules to the pili. Using this technique, Lawn & Meynell (1970) distinguished four serotypes in the F-like class of pili and two in the I-like class. The validity of their classification was confirmed by Harden & Meynell (1972), employing a method that depended on the ability of pilus antibody to inhibit genetic transfer in a specific manner. The present communication is concerned with sex pili as immunogens. Chickens were vaccinated with live cultures of *Escherichia coli* K12 possessing one or other of three highly efficient transfer factors: F (Lederberg, Cavalli & Lederberg, 1952; Hayes, 1953), I (Ird16; Hardy *et al.* 1973) and A2 (Smith & Heller, 1973). Their immunity against salmonella strains in which F or I had been established was then challenged. It was difficult to prepare suitable challenge strains, because cultures had to contain enough piliated bacteria for them to be susceptible to visible lysis by pilus-specific phages – the MS2 phage (Davis, Strauss & Sinsheimer, 1961) in the case of F⁺ strains and the If 1 phage (Meynell & Lawn, 1968) in the case of I⁺ strains. Some of the strains satisfying this criterion exhibited too great a reduction in pathogenicity; in others, the transfer factors were unstable. Eventually an F⁺ strain of *Salmonella heidelberg* and an I⁺ strain of *Salmonella typhimurium* (phage type 36) were obtained which appeared suitable. An F⁺ form of the *S. typhimurium* strain was also used in some experiments although cultures of this strain were not lysed so completely by the MS2 phage as were cultures of the F⁺ *S. heidelberg* strain.

METHODS

Bacterial strains and cultural conditions. Unless stated, in none of the *E. coli* or salmonella strains employed had either transfer factors or known transmissible characters been detected. Cultures were maintained on Dorset's egg medium at 5 °C. When required as broth cultures they were inoculated into 10 ml nutrient broth (Oxoid No. 2) in 1 oz Universal containers and incubated at 37 °C for 24 h in a sloped position. The cultures contained approximately 10⁹ viable organisms/ml.

Preparation of vaccinal and challenge strains. The vaccinal strains were prepared by establishing F, I and A2 in a nalidixic acid-resistant mutant (*nal^r*) of a *lac⁻* auxotrophic *E. coli* K12 strain. Proof of establishment was susceptibility to the F-specific phage, MS2, or the I-specific phage, Ifi, in the case of the F⁺ and I⁺ strains, and ability to mobilize resistance determinants by the technique of Smith & Linggood (1970) in the case of the A2⁺ strain. For the preparation of challenge strains, auxotrophic *E. coli* K12F⁺Tet⁺ and I⁺Tet⁺ strains were first prepared by using F and I to mobilize Tet in a Tra⁻ *E. coli* K12 strain and to transfer it to the *nal^r* K12 strain (Tet, tetracycline resistant determinant described by Smith & Gyles, 1970; Tra, transfer factor). The auxotrophic *E. coli* K12F⁺Tet⁺ strain and a *S. heidelberg* strain were then grown together in nutrient broth, the mixed culture centrifuged and the deposit inoculated on to plates of medium containing (g/l): K₂HPO₄, 7; KH₂PO₄, 3; (NH₄)₂SO₄, 1; MgSO₄·7H₂O, 0·1; NaCl, 5; glucose, 5; agar, 15; and tetracycline, 0·020. Colonies of *S. heidelberg* isolated on this medium after incubation at 37 °C for 48 h were purified by replating. From these, broth cultures were prepared and a loopful spread evenly over small areas of plates of tryptose agar (Difco). A drop of an undiluted preparation of phage MS2 was placed on each dried, inoculated area. After incubation for 8 h at 37 °C, the plates were examined for phage lysis. The parent broth cultures of those tryptose agar cultures that appeared to have been completely lysed and that were smooth as judged by the acriflavine slide test (Braun & Bonestell, 1947) were examined further, to find one that was lethal for chickens and that was still fully susceptible to the MS2 phage when isolated from chickens it had killed. An F⁺Tet⁺ strain of *S. typhimurium* was prepared in a similar manner to the F⁺Tet⁺ strain of *S. heidelberg*, the particular strain selected being slightly less susceptible to the MS2 phage than the *S. heidelberg* strain. An I⁺ challenge strain was prepared from the *S. typhimurium* strain in the same manner as the F⁺ challenge strains except that the donor strain was I⁺Tet⁺ instead of F⁺Tet⁺ and the phage was Ifi instead of MS2.

Vaccination and challenge procedure. Groups of Light Sussex chickens from a salmonella-free flock were injected intravenously (i.v.) with 0·5 ml broth culture of one or other of the three vaccinal strains, or of the *E. coli* K12 strain from which they were prepared, when they were two weeks and again when three weeks of age. Thirteen days after the second injection the chickens were injected i.v. with 0·5 ml broth culture of one of the salmonella challenge strains. A group of unvaccinated chickens was challenged at the same time. The number of chickens in each group that died on each of the first 9 days after challenge was recorded; few deaths that could be directly related to the infection occurred after this time. Samples from the livers of most of the chickens that died were cultured on plates of MacConkey's agar and ten colonies from each plate examined for susceptibility to the MS2 phage or the Ifi phage.

Statistical analysis. The results of the lethality tests were analysed in 2 × 2 contingency tables and examined for significance by the χ^2 test by Mr J. G. Rowell, A.R.C. Statistics Group, Cambridge University.

Antisera. These were prepared against the vaccinal strains by the multiple i.v. injection of rabbits with live broth cultures.

RESULTS

Mortality following infection

Compared with vaccination with the Tra⁻ strain, vaccination with the F⁺ strain, but not the I⁺ or A2⁺ strains, of *E. coli* K12 significantly reduced the mortality following infection with an F⁺ strain of either *S. heidelberg* or *S. typhimurium* (Tables 1 and 2). Likewise,

Table 1. *The effect of vaccinating chickens with an F⁺, I⁺ or A2⁺ strain of E. coli K12 on subsequent infection with an F⁺ strain of S. heidelberg*

For details, see text.

Vaccinal strain	No. of chickens vaccinated		Percentage that died when infected	
	Expt. 1	Expt. 2	Expt. 1	Expt. 2
<i>E. coli</i> K12F ⁺	43	40	23.3‡	22.5‡
<i>E. coli</i> K12I ⁺	—	40	—	55.0 NS
<i>E. coli</i> K12A2 ⁺	—	40	—	70.0 NS
<i>E. coli</i> K12Tra ⁻	39	40	64.1	60.0
None	40	40	67.5 NS	95.0‡

Significance compared with the results for the *E. coli* K12Tra⁻ group: NS, not significant ($P > 0.05$); *, $0.01 < P < 0.05$; †, $0.001 < P < 0.01$; ‡ $P < 0.001$.

Table 2. *The effect of vaccinating chickens with an F⁺, I⁺ or A2⁺ strain of E. coli on subsequent infection with an F⁺ or I⁺ strain of S. typhimurium*

For details, see text.

Vaccinal strain	No. of chickens vaccinated			Percentage that died when infected with:		
				F ⁺ strain	I ⁺ strain	
	Expt 1	Expt 2	Expt 3	Expt 1	Expt 2	Expt 3
<i>E. coli</i> K12F ⁺	25	24	44	54.0‡	29.2‡	34.1 NS
<i>E. coli</i> K12I ⁺	—	—	46	—	—	15.2‡
<i>E. coli</i> K12A2 ⁺	—	—	47	—	—	19.1*
<i>E. coli</i> K12Tra ⁻	22	28	46	95.4	82.1	45.7
None	25	30	46	100.0 NS	90.0 NS	80.4‡

For significance, see Table 1.

vaccination with the I⁺ or A2⁺ strain, but not the F⁺ strain, of *E. coli* K12 significantly reduced the mortality resulting from infection with an I⁺ strain of *S. typhimurium* (Table 2). In experiment 2 (Table 1) and experiment 3 (Table 2) the mortality rate in the group of chickens vaccinated with the Tra⁻ strain of *E. coli* K12 was significantly less than in the unvaccinated group.

In the majority of cases, all of the ten colonies isolated on MacConkey's agar from the livers of vaccinated and unvaccinated chickens that had died following infection with the F⁺ strains of *S. heidelberg* and *S. typhimurium* were fully susceptible to the MS2 phage. Usually, half or more of the ten colonies examined from each of the chickens that died within three days of infection with the *S. typhimurium* I⁺ strain were susceptible to the phage, but most colonies from chickens that died after this time were not.

Slide agglutination tests

Using undiluted antisera, the F⁺ strain of *S. heidelberg* was agglutinated by the F⁺ strain of *E. coli* K12 antiserum but not by antisera to the I⁺ or A2⁺ strains of *E. coli* K12. The I⁺ strain of *S. typhimurium* was agglutinated by an antiserum to the I⁺ and A2⁺ strains, but not to the F⁺ strain, of *E. coli* K12.

DISCUSSION

The present investigation was undertaken as a prelude to the immunological study of transmissible pathogenic characters. Because of the poor selection methods that exist for their detection, the characters available for study are usually those whose efficiency of transfer is high, probably approaching that of F, I and A2. Because efficiency of transfer and degree of piliation are directly related, the immunity produced by the sex pili determined by the transfer factors of some of these pathogenic characters might, therefore, be of the order of that produced by F, I and A2 in the present investigation. If so, it would be more than sufficient to complicate some aspects of the immunological study of these characters. Should the genes determining such characters and pilus production be located on the same plasmid this complication would be difficult to overcome.

Whether sex pilus immunity is important under natural conditions is another matter. Most transfer factors apparently exist in a repressed state and, as a consequence, many Tra⁺ bacteria may produce too few pili either to provoke a significant level of antibody or to react with such antibody in potential disease-producing situations.

The I⁺ and A2⁺, but not the F⁺, strains of *E. coli* K12 produced an immunity against the I⁺ challenge strain of salmonella, and the F⁺ strain, but not the I⁺ and A2⁺ strains, of *E. coli* K12 produced an immunity against the F⁺ challenge strain of salmonella; these results are in agreement with the results of the agglutination tests. Our inability to demonstrate a relationship between F and I by either method confirms the observations of earlier workers using agglutination and other techniques to detect antibody.

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REFERENCES

- BRAUN, W. & BONESTELL, A. E. (1947). Independent variations of characteristics in *Brucella abortus* variants and their detection. *American Journal of Veterinary Research* **8**, 386-390.
- DAVIS, J. E., STRAUSS, J. H., JUN. & SINSHEIMER, R. L. (1961). Bacteriophage MS2: another RNA phage. *Science, New York* **134**, 1427.
- HARDEN, V. & MEYNELL, E. (1972). Inhibition of gene transfer by antiserum and identification of serotypes of sex pili. *Journal of Bacteriology* **109**, 1067-1074.
- HARDY, K. G., MEYNELL, G. G., DOWMAN, J. E. & SPRATT, B. G. (1973). Two major groups of colicin factors; their evolutionary significance. *Molecular and General Genetics* **125**, 217-230.
- HAYES, W. (1953). Observations on a transmissible agent determining sexual differentiation in *Bacterium coli*. *Journal of General Microbiology* **8**, 72-88.
- LAWN, A. M. & MEYNELL, E. (1970). Serotypes of sex pili. *Journal of Hygiene* **68**, 683-694.
- LEDERBERG, J., CAVALLI, L. L. & LEDERBERG, E. M. (1952). Sex compatibility in *E. coli*. *Genetics* **37**, 720-730.
- MEYNELL, G. G. & LAWN, A. M. (1968). Filamentous phages specific for the I sex factor. *Nature, London* **217**, 1184-1186.
- ØRSKOV, I. & ØRSKOV, F. (1960). An antigen termed f⁺ occurring in F⁺ *E. coli* strains. *Acta pathologica et microbiologica scandinavica* **48**, 37-46.
- SMITH, H. WILLIAMS & GYLES, C. L. (1970). The relationship between different transmissible plasmids introduced by F into the same strain of *Escherichia coli*. *Journal of General Microbiology* **62**, 277-285.
- SMITH, H. WILLIAMS & HELLER, E. D. (1973). The activity of different transfer factors introduced into the same plasmid-containing strain of *Escherichia coli* K12. *Journal of General Microbiology* **78**, 89-99.
- SMITH, H. WILLIAMS & LINGGOOD, M. A. (1970). Transfer factors in *Escherichia coli* with particular regard to their incidence in enteropathogenic strains. *Journal of General Microbiology* **62**, 287-299.