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Antibody induced elimination of the plasmid controlled K88 adhesion factor from a porcine enteropathogen

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Summary. The heat labile K88 antigen which is a product of an episomal gene, was eliminated from a porcine enteropathogen by passage through media containing sow colostrum antibodies raised against heat stable antigens of *E. coli*. In consequence the porcine enteropathogen lost its ability to adhere to and agglutinate chicken erythrocytes, a model system for piglet intestinal adhesion and virulence.

INTRODUCTION

During recent tests on the efficacy of an oral *E. coli* vaccine 'Intagen', which is administered in the feed, investigations were made on the response of weaned pigs to infection by an 0149 : K91, K88ac strain of *E. coli*. In addition to the significant benefits in health and performance previously reported (Porter, Kenworthy & Allen, 1974) it was observed that in the orally immunized, but not control animals, changes occurred in the surface antigens of the infecting strain. The immunized animals were shown to be excreting K88$^-$ variants of the *E. coli* strain which in turn proved to be ineffective in establishing infection when administered to further control animals (Porter, Parry & Allen, 1977).

These observations were somewhat surprising since the response was generated against a heat inactivated preparation and antibody responses to the heat stable 0 antigens were expected. Furthermore, the ability of antibodies to effect elimination of genetic elements responsible for the synthesis of surface virulence determinants is hitherto unrecorded, although plasmid curing can be achieved by a number of chemical agents (Mitchell & Kenworthy, 1977; Salisbury, Hedges & Datta, 1972). This provides an attractive new concept that the protective mechanisms attributable to intestinal antibody include the elimination of virulence determinants from wild enteropathogens locally in the gut. We therefore examined this further using colostrum from an immunized sow in order to establish its validity under controlled conditions *in vitro*.

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MATERIALS AND METHODS

E. coli

A porcine enteropathogen, strain L2 (08 : K87, K88ab) carrying the Hly and K88 plasmids, was used to examine the effects of antibody on K88 production in culture.

Whey samples

Colostrum was collected 2 h after parturition from a sow that had been given *E. coli* vaccine by a combina-

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tion of oral and parenteral routes. The vaccine was composed of heat inactivated aqueous suspensions of seven known porcine enteropathogens belonging to serotypes with 0 antigens 08, 045, 0138, 0139, 0141, 0147 and 0149 (Porter, Kenworthy, Holme & Horsfield, 1973).

The immune whey was separated and kept at −20° until required. Milk from a non-vaccinated animal was used as a source of normal whey. The whey samples were checked for sterility before use and filtration was found to be unnecessary.

Experimental procedure

Two millilitre aliquots of nutrient broth (Oxoid CM1) containing 2–10% of sterile milk whey or immune colostral whey were prepared. Strain L2 was then passaged seven times through the broths, each time 0·02 ml of the culture was transferred and growth continued for 24 h at 37° before the next passage. Following each passage 0·02 ml of culture was inoculated into 9 ml of nutrient broth and grown for a further 24 h at 37°. The bacteria were then harvested by centrifugation, resuspended in 1 ml sterile saline and the suspension heated at 60° for 30 min to release the K88 antigen. The level of K88 in each culture was then assayed using the chicken erythrocyte method of Parry & Porter (1977). The K88 antigen of porcine enteropathogenic strains of *E. coli* exhibits specific affinity for the membranes of chicken erythrocytes and gives rise to agglutination of the cells. This provides a useful assay system for measuring the level of K88 antigen in a culture. K88 was not titrated in the presence of the whey samples as it was found that factors present in them led to the masking of the antigen.

The passaged cultures were plated on blood agar to confirm that contamination by other bacteria had not occurred. The plates were examined for the presence of non-haemolytic variants of strain L2 and also single colonies were checked for the presence of the K88ab antigen in slide agglutination tests.

RESULTS

Effect of passaging on the K88 plasmid

The counts done on all the passaged cultures after growth for 24 h in 9 ml nutrient broth gave similar results, hence the K88 titre for the whole cultures gave a reliable indication of the proportion of $K88^+$ bacteria present.

The level of K88 production was unaffected by up to five passages (120 h growth) in plain broth or in broth containing normal milk whey. However, after the fifth passage the K88 titres began to fall and this was found to be associated with an increasing number of 'rough colonies' shown by slide agglutination. In contrast three passages (72 h growth) through broth containing 3–10% of immune whey brought about a dramatic decline in K88 titres and by the fifth passage K88 antigen could no longer

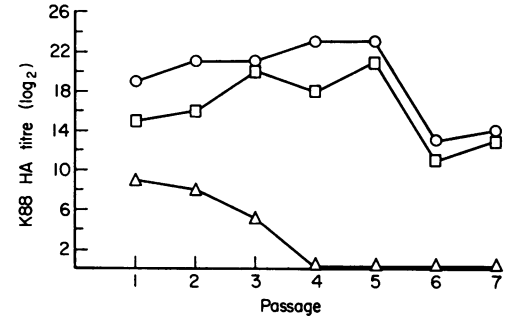

Figure 1. Antibody elimination of adhesion determinants. (○), Nutrient broth; (□), broth + normal whey; (△), broth + immune whey.

be detected by the assay (Fig. 1). The effect of the immune whey was therefore most apparent after four or five passages as by that time strain L2 passaged in the presence of the immune whey had completely lost its K88 antigen while the strain passaged in the presence of normal whey was still smooth and was producing large amounts of K88 antigen.

Results obtained on testing individual colonies from the passaged cultures with K88 antiserum correlated well with the titration results for the whole culture, for example the twenty colonies tested after growth of cultures for 72 h in plain broth, or broth containing 10% of normal whey were all $K88^+$, while the ten colonies tested from the culture grown for 72 h in broth containing 10% of immune whey were all $K88^-$.

Effect of passaging on the Hly plasmid

Strain L2 also contained the Hly plasmid and so the passaged cultures were examined for loss of this character. A few nonhaemolytic clones of strain

L2 were obtained from the cultures passaged through the broths containing immune, but not normal whey. However, the effect was not very marked.

DISCUSSION

The protective value of colostrum against porcine neonatal *E. coli* enteritis is well recognized. There is no transplacental transfer of maternal immunoglobulin '*in utero*' and the neonate depends upon the intestinal absorption of high levels of maternal colostral immunoglobulin during the first few days of its life for survival. There are dramatic changes in the maternal lactation profile at the beginning of lactation which are associated with the physiological requirement for the transfer of passive immunity (Porter, Noakes & Allen, 1970). Over a period of a few days there is a rapid decline in the immunoglobulin level in the mammary secretions so that the level in the later milk is less than 5% of that in the colostrum. In the investigation described here milk whey was used as the control secretion as it contained the components of mammary origin other than the antibodies which are selected for in the transudatory mechanisms of colostrum formation.

In neonatal colibacillosis the K88 antigen displays specific characteristics of attachment for porcine intestinal epithelium and elimination of the genetic elements responsible for its synthesis in an enteropathogenic strain results in loss of virulence (Smith & Linggood, 1971). K88 antibodies can protect young pigs from infection (Rutter & Jones, 1973) and can block adhesion of K88$^+$ *E. coli* to porcine intestinal membranes (Wilson & Hohmann, 1974; Sellwood, Gibbons, Jones & Rutter, 1975). However, in relation to the present observations it should be emphasized that K88 is a heat labile antigen and the *E. coli* vaccine used in the production of the immune whey was comprised of heat stable antigens; furthermore, anti-K88 antibodies were undetectable. The curing phenomenon described is therefore unassociated with anti-K88 activity as it is presently understood.

The loss of the ability to synthesize the K88 antigen was clearly due to the permanent loss of the controlling gene and not to the temporary suppression of its effect. The titration was performed on cultures that had grown for 24 h in the absence of immune whey representing many generations of bacterial growth through which loss of the character had remained stable. Permanent loss of a plasmid controlled character is normally accepted as evidence of plasmid curing.

These findings are intriguing as bacterial modification is normally disadvantageous to the host. In this case, however, genetic changes occur resulting in antigenic modification of the pathogen which moves the balance of the relationship strongly in favour of the host. This antibody mediated activity is clearly of considerable significance in terms of herd health as it gives rise to a reduction in the virulence of the enteropathogenic bacteria present in the environment.

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