Pathogenicity of Clostridium perfringens for Germ-Free Guinea Pigs After Oral Ingestion

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Received for publication 10 November 1969

Germ-free guinea pigs died with signs and lesions of acute enterotoxemia after oral ingestion of C. perfringens types B, C, D, and E. The signs and lesions observed resembled those seen in acute enterotoxemia of sheep and cattle and the naturally occurring disease seen in "ex-germ-free" guinea pigs. C. perfringens type A was found to be innocuous. Conventional guinea pigs did not become ill after ingestion of any of the five toxigenic types.

The germ-free guinea pig has become a useful model in the study of infectious diseases (6, 7, 8). However, it has been our experience, as well as that of others, that these animals are difficult to maintain in conventional quarters after their removal from the germ-free environment. Within several days after being placed in an ordinary animal facility, many of them develop a severe intestinal distention and within a few hours a flaccid paralysis occurs which is followed by death. In a previous study, Clostridium perfringens type E was isolated from such a group of affected "ex-germ-free" guinea pigs (submitted for publication). When broth cultures of this organism were fed to germ-free guinea pigs, typical signs and lesions of acute enterotoxemia and death were produced. This report describes the effects of oral feeding of other selected strains of C. perfringens to germ-free and conventional guinea pigs.

MATERIALS AND METHODS

Hartley strain guinea pigs were used in this study. Newborn germ-free guinea pigs were obtained by Caesarean section of full-term pregnant females supplied by the Rodent and Rabbit Production Section of the National Institutes of Health. The techniques for maintaining germ-free animals and microbiological procedures for monitoring the germ-free system for bacterial and fungal contamination have been described previously (4). From the same colony, comparable-size (200 g) conventional guinea pigs were obtained and maintained in a laboratory animal room with food and water given ad libitum.

The various toxigenic types of C. perfringens employed in this study are listed in Table 1. In addition to the five prototypes obtained from the National Communicable Disease Center, two other strains were included: C. perfringens type A, supplied by Bruce Phillips, Laboratory of Microbial Immunity, National Institute of Allergy and Infectious Diseases; and C. perfringens type E, as previously described in the study cited above. The cultures were maintained in a standard Brain Heart Infusion broth (Difco) containing cooked meat particles and incubated in an atmosphere of 95% N and 5% CO. They were examined for purity prior to use.

Germ-free and conventional guinea pigs were exposed to the various types of C. perfringens by feeding the organisms in their water supply. Five-milliliter amounts of an actively growing 24-hr broth culture were diluted in 100 ml of water. This served as the sole source of drinking water for these animals. The germ-free contact control animals were kept in another cage within the same monocontaminated isolation unit but furnished a separate food and water supply. Except for being housed in a standard animal room, the conventional control animals were maintained in a similar manner. The procedure for recovering and identifying C. perfringens from animals at necropsy has been described previously (submitted for publication).

RESULTS

The mortality of the Hartley strain guinea pigs fed the various toxigenic types of C. perfringens is indicated in Table 1. All germ-free guinea pigs except those exposed to type A died within 12 to 96 hr after the culture was placed in the water bottles. The germ-free control animals lived about 12 hr longer than the inoculated guinea pigs. Organisms were readily recovered from the intestinal contents, blood, and liver of the dead animals, as well as the surfaces throughout the monocontaminated isolation unit. No ill effects were ob-

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TABLE 1. Mortality of Hartley strain guinea pigs after oral ingestion of toxigenic types of Clostridium perfringens

| Designation   | Germ-free | Conventional |
|---------------|-----------|--------------|
|               | Inoculated | Contact      | Inoculated | Contact |
| Type A-no. 1434 | 0/6*      | 0/2          | 0/12       | 0/4     |
| Type A-Phillips | 0/4       | 0/4          | 0/4        | 0/4     |
| Type B-KA41    | 2/2       | 2/2          | 0/4        | 0/4     |
| Type C-KA42    | 3/3       | 2/2          | 0/4        | 0/4     |
| Type D-KA43    | 3/3       | 3/3          | 0/4        | 0/4     |
| Type E-99      | 4/4       | 2/2          | 0/4        | 0/4     |
| Type E-Ex-germ-free | 12/12 | 8/8      | 0/12       | 0/6     |

* Number of animals dying/number exposed.

observed after feeding of *C. perfringens* type A to germ-free guinea pigs nor were signs of illness observed after feeding any of the five types to conventional guinea pigs.

The gross signs and lesions observed in the experimentally infected germ-free guinea pigs were similar to those seen in naturally infected "ex-germ-free" guinea pigs. Death was relatively rapid, with the signs being limited to ruffled hair, progressive lethargy, and a flaccid paralysis. In the more protracted cases some diarrhea was present. At necropsy the lungs and brain appeared normal, the liver was swollen and friable, the spleen was swollen and cyanotic in appearance, and the kidneys were pale. The appearance of the intestinal tract varied among the animals from normal to slightly hyperemic, with the exception of the cecum which was hemorrhagic, greatly distended, and contained a foul-smelling gas. In histopathological studies some edema was found in the lungs, and cloudy swelling, hyperemia, and congestion were the predominant lesions seen in the livers, spleens, and kidneys. The cecum was edematous and hemorrhagic, and sloughing of the mucosa was common. Throughout the rest of the digestive tract, slight edema and hyperemia were observed.

DISCUSSION

The susceptibility of the germ-free guinea pig and the tolerance of the conventional guinea pig to *C. perfringens* B, C, D, and E after oral ingestion were demonstrated. In addition to an immunological deficit, the germ-free guinea pig differs from the conventional animal in other respects which must be considered in assessing the difference in susceptibility. The lining of the intestinal tract of the germ-free animal is much thinner than that of the conventional guinea pig, and perhaps this barrier is more easily breached by toxins and bacteria than that of the conventional animal. Further, the cecum of the germ-free guinea pig is greatly enlarged and the contents are quite viscous. As a result there is presumably a certain degree of stasis of the intestinal flow in this area. Upon necropsy the lesions in the ceca were more striking than those found elsewhere in the intestinal tract. The absence of competitive organisms may have allowed unrestrained growth of *C. perfringens* with consequent production of large amounts of toxin, devitalization of tissue, and bacterial invasion of the tissues. When one considers the relatively slight invasive tendency of *C. perfringens* in conventional animals, it is difficult to ascribe the lethal effect in the germ-free guinea pig entirely to the absence of preformed specific and nonspecific antibodies.

The two strains of *C. perfringens* type A used in this study were not pathogenic by the oral route for Hartley strain germ-free guinea pigs. This was not surprising, since type A enterotoxemia seems to be limited primarily to man. Canada and Strong (1) reported that three strains of *C. perfringens* isolated from human enteritis were not pathogenic when fed to germ-free mice. Although early studies by Nygren (5) indicated that rhesus monkeys were affected by oral ingestion of type A, Weiss et al. (9) later concluded that type A was not pathogenic by the oral route for rhesus monkeys or mice. Hauschild et al. (2) found that all five of the type A strains isolated by them produced enteritis in young lambs. Their studies led them to suggest that type A strains were also responsible for spontaneous diarrhea in young lambs; however, they could not prevent the disease syndrome by immunization with anaculture, nor did they feel that the alpha toxin was responsible for the signs and lesions observed (3). The tolerance of the germ-free guinea pig to type A has also been demonstrated by Phillips and Gorstein (6) who used this type for a potentiating organism for *Entamoeba histolytica* infection.

Our study shows the germ-free guinea pig to be susceptible to the same toxigenic types of *C. perfringens* that cause disease in other animals. The pathogenesis of the virulent animal strains is poorly understood. The germ-free guinea pig, whose diet can be varied and whose bacterial flora can be initiated and qualitatively controlled, may prove to be a suitable experimental host for determining the pathogenesis of *C. perfringens* enterotoxemia.

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