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Antigenic Cross-Reaction Between Mouse Intestine and a Member of the Autochthonous Microflora

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Antigenic cross-reaction has been demonstrated between homogenates of intestine taken from fetal mice and suspensions of a *Bacteriodes* species isolated from the large intestines of normal adult mice. This observation provides an explanation for the lack of immunological response observed in mice after antigenic stimulation with this member of the normal autochthonous intestinal flora of these animals.

In a previous publication (5), mice were shown to be unresponsive to antigenic challenge with suspensions of a *Bacteroides* sp. found as members of the autochthonous microflora of the rodent gastrointestinal tract. However, the same bacterial suspension proved to be highly immunogenic in sheep, rabbits, and guinea pigs. It was proposed that similarity between antigens of *Bacteroides* and some components of mouse tissues might account for this seemingly anomalous state which has been termed "immunological indifference." This communication presents evidence of antigens shared between a species of *Bacteroides* isolated from the large bowels of adult mice and the intestinal tissues of neonatal mice.

MATERIALS AND METHODS

Animals. Mice were obtained from a specific, pathogen-free colony of outbred animals maintained in the School of Microbiology, University of New South Wales, Kensington, Australia.

Conventionally raised sheep, guinea pigs, and rabbits were supplied by the Animal Breeding and Holding Unit of the University.

Bacterial cultures. The following strains of organisms were isolated from the intestinal contents of mice: *Bacteroides* sp. (no. B22) and *Escherichia coli* (no. E1). The *Bacteroides* sp. was maintained on a medium similar to that described by Schaedler et al. (8), except that 6% horse blood was added instead of hemin solution. The *E. coli* strain was subcultured on nutrient agar (Oxoid).

Preparation of tissue homogenates: (i) prenatal intestinal tissue. Rabbit, guinea pig, and mouse fetuses were taken from their mothers by caesarian section to ensure freedom from bacterial antigens. These animals were killed by spinal dislocation and the intestines were removed. The intestines were washed gently in sterile saline to remove any blood. The tissue was weighed and homogenized in a small volume of saline with the aid of a Teflon grinder. The approximate concentration of these homogenates was 500 mg of tissue per ml.

(ii) Other tissues. Hearts, kidneys, and livers were removed from adult mice, suspended in a small volume of sterile saline, and homogenized with a Teflon grinder. The concentration of these tissues was approximately 500 mg of tissue per ml.

Immunization schedules: (i) bacterial vaccines. Heat-killed organisms were used for inoculating the animals parenterally. The organisms were grown on appropriate media for 24 to 48 h at 37 C, suspended on 0.85% saline, and heated at 80 C for 30 min. After being washed three times in saline, suspensions containing 10^9 organisms per ml were prepared in this medium.

Rabbits were inoculated with 10^8 organisms, whereas the inoculum for sheep was 10^9. All animals were given three injections at weekly intervals and then were bled 7 days after the last injection. The sera were collected after clotting and centrifugation of the blood samples and, if necessary, stored at −70 C.

(ii) Intestinal antigens. Rabbits were inoculated once a week for 6 weeks with a suspension of mouse intestinal homogenate, commencing with a dose of 2.5 mg and increasing up to 80 mg.

Absorption of immune serum: (i) bacterial suspensions. Washed suspensions of bacterial cells containing 10^{10} organisms per ml were used. These suspensions were dispensed in 1-ml volumes into centrifuge tubes. After centrifugation, the supernatant was removed and replaced by 1 ml of undiluted immune serum. The serum was incubated with the bacteria for 1 h at 37 C and then collected after centrifugation. Three adsorptions were carried out on each serum sample.

(ii) Tissue suspensions. A volume (1 ml) of intestinal homogenate (containing 5 mg of tissue per ml) was centrifuged at 4,000 rpm for 30 min, and the supernatant was discarded. Serum was added to the tissue and incubated for 1 h at 37 C. This absorption was repeated three times.

Serological techniques. Complement-fixation ti-

ters were determined by the methods of Cruickshank (3). The following concentrations of antigen were used for these titrations: organisms, 10^9/ml; tissues, 25 mg/ml. Because of the varied nature of the antigenic suspensions used, special care was taken to determine whether they possessed any anticomplementary activity.

RESULTS

Three sheep were immunized with killed suspensions of *Bacteroides* B22 which had been isolated from mouse intestine. Each of the three antisera was then tested for complement-fixing antibody against *Bacteroides* antigens before and after absorption with suspensions of neonatal intestinal tissue derived from different animal species (Fig. 1). The most significant reductions in titer occurred when mouse intestinal tissue was used for absorption, varying from four- to sixfold in the three antisera. Whereas a two- to threefold reduction was found in the sera absorbed with the intestinal tissue homogenates from rabbits, neither guinea pig intestine nor *E. coli* absorptions significantly affected the serum titers. Most antibody was removed after absorption with the homologous antigen suspension.

To ensure that this result was specific for the mouse intestinal tissue, antiserum from the same sheep was absorbed with suspensions of other tissues from neonatal mice. Intestinal homogenate was the only preparation which removed a significant amount of anti-*Bacteroides* antibody (Table 1). None of the tissues was anticomplementary.

Further control experiments included investigation of antisera produced in sheep against suspensions of *E. coli*. Complement-fixation titers against this organism after absorption with the various intestinal homogenates are shown in Fig. 2. No significant reduction in titer was observed after absorption with any of the suspensions except for the homologous antigen preparation.

In a second series of experiments, groups of rabbits were immunized with *Bacteroides* or mouse intestinal antigen. Antisera from these rabbits were tested for complement-fixing antibody by using *Bacteroides* or mouse intestine suspensions. One high-titered antiserum from each group was then absorbed with either the bacterial suspension or the gut tissue before complement-fixation tests with both these antigens (Table 2). Each antigen induced high titers of complement-fixing antibody which was readily absorbed by the homologous antigen. More-

TABLE 1. *Complement-fixation titer of a sheep antiserum against Bacteroides antigen after absorption with various mouse tissue homogenates*

Material used for absorption	Complement-fixation titer against *Bacteroides* sp.
Nil	15,360
Mouse heart[a]	15,360
Mouse liver	7,680
Mouse kidney	15,360
Mouse red blood cells	7,680
Mouse intestine	480

[a] All suspensions were tested for anticomplementary activity.

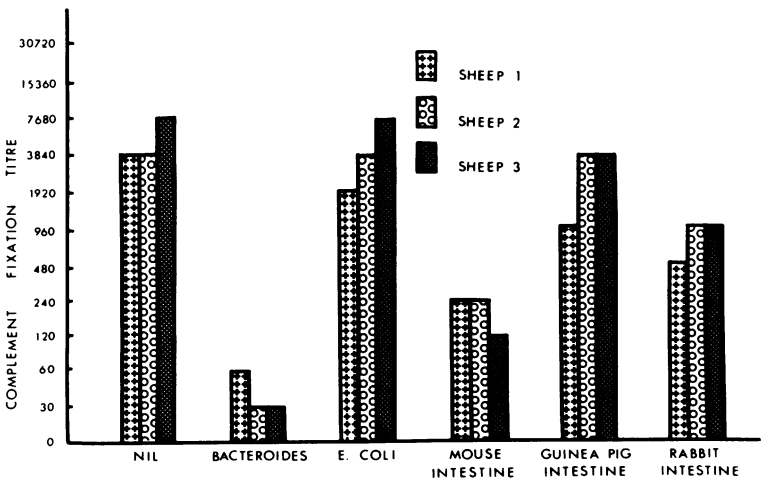

FIG. 1. *Complement-fixation titers of sheep antisera against Bacteroides sp. after absorption with Escherichia coli or tissue homogenates. Bacteroides antigen was used to produce the antiserum and also as the antigen in the complement-fixation test. The Bacteroides species was originally isolated from mouse intestine.*

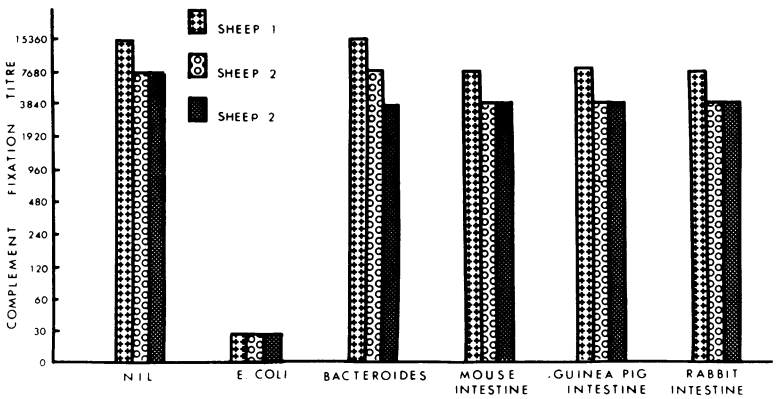

FIG. 2. *Complement-fixation titers of sheep antisera against Escherichia coli after absorption with Bacteroides sp. or tissue homogenates. Escherichia coli antigen was used to produce the antiserum and also as the antigen in the complement-fixation test. The Escherichia coli strain was originally isolated from mouse intestine.*

over, absorption with intestinal antigen removed significant amounts of *Bacteroides* antibody; similarly, *Bacteroides* antigen absorbed the antibody against the mouse intestine. Significantly, each antigen suspension induced formation of low levels of antibody which reacted with the other antigen.

The specificity of this reaction was investigated further in absorption studies of rabbit antisera with other suspensions. Thus, in Fig. 3 are shown the complement-fixation titers against mouse intestinal antigen of rabbit anti-intestine sera after absorption with different suspensions. Only absorption with *Bacteroides* antigen and the homologous antigen suspension resulted in significant removal of anti-intestine antibody. Similar results were found with anti-*Bacteroides* antisera from rabbits.

TABLE 2. *Complement-fixation titers of rabbit sera immunized with mouse intestinal antigen or Bacteroides sp.*

Antigen used for immunization	Antigen used for absorption	Complement-fixation titers	
		Bacteroides sp.[a]	Mouse intestine[a]
Bacteroides sp.	Nil	7,680	120
	Bacteroides sp.	60	<30
	Mouse intestine	1,920	<30
Mouse intestine	Nil	480	15,360
	Bacteroides sp.	<30	120
	Mouse intestine	30	120
Normal serum	Nil	60	30

[a] Antigen used.

DISCUSSION

In a previous publication (5), it was shown that rodents are completely unresponsive to immunological challenge with suspensions of *Bacteroides* organisms, a member of the autochthonous intestinal flora. That this organism is a good antigen was shown in experiments with sheep.

Other workers have reported low immunogenicity of nonpathogenic members of the indigenous flora in their natural host (2). David et al. (4) examined spiral organisms closely associated with the cecal mucosa of the rat and observed occasional penetration of these bacteria into the tissue with no noticeable effect on the host. These authors speculated that the host is immunologically tolerant to some members of the normal flora. The findings described above suggest the mechanism whereby such a state of unresponsiveness is achieved. Anti-*Bacteroides* antibody was removed from an antiserum by adsorption with neonatal mouse intestine. Absorption with other mouse tissues or intestinal homogenates had no effect except for some reduction with rabbit intestine. The use of neonatal tissue was an important feature of these experiments since it eliminated the possibility of any residual bacterial antigens in the tissue homogenates. Similar experiments showed that anti-mouse intestine antibody could be removed by absorption with *Bacteroides* suspensions. Thus, strains of *Bacteroides*, which are members of the mouse autochthonous flora, share antigens with mouse

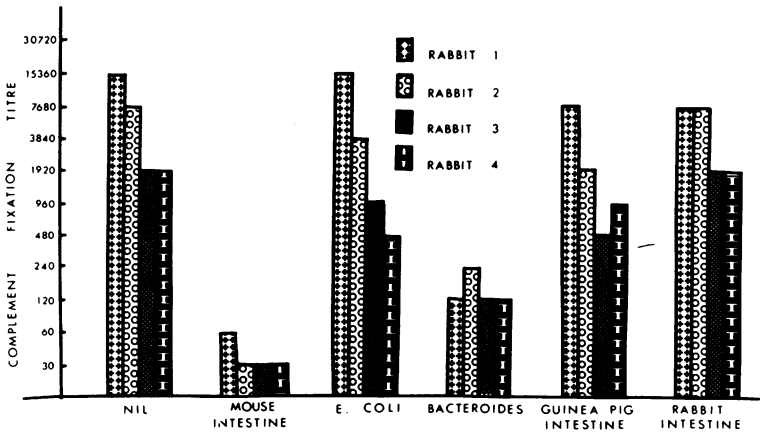

FIG. 3. *Complement-fixation titers of rabbit antisera against neonatal mouse intestine after absorption with Bacteroides sp., Escherichia coli, or tissue homogenates. The intestinal antigen was used to produce the antiserum and also as the antigen in the complement-fixation test.*

intestinal tissues. Since the mouse is tolerant to all the exposed antigens of its own tissues, it will also be unresponsive to these bacterial antigens. The term immunological indifference is preferred for this phenomenon since it has been shown that the mouse has a level of "natural antibody" against *Bacteroides* antigen. The levels of these antibodies are not boosted after parenteral injection of the antigen (5).

The lack of immunological response to the *Bacteroides* sp. antigens may contribute to colonization of the normal intestine by large numbers of these bacteria. Although immunological mechanisms have been shown to act in the intestinal lumen in certain cases, e.g., copro antibodies in patients with cholera (6) and in swine with colibacillosis (1), there is no evidence that antibodies influence the populations of bacteria present in the bowel of normal animals. Further studies are needed on the immune response of these animals against other members of the autochthonous flora to determine whether this unresponsiveness is a general phenomenon. A study of the autochthonous spiral organisms of the rodent would be of special interest because of their close association with the intestinal mucosa (3, 6).

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