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Intestinal Resection and Anastomosis in Neonatal Gnotobiotic Piglets

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We describe a surgical method for ileal resection and anastomosis in newborn germfree piglets that was undertaken to establish a model that can be used for immunologic research and other applications. A preliminary experiment indicated that neonatal piglets with resection of approximately 60 cm of their ileum (removal of approximately 90% of the continuous ileal Peyer patches; group A) and those in which the ileum was transected (group B) could be maintained germfree for 35 d, colonized with defined gut flora, and maintained in a clean room until 70 d of age. In the final study, 12 piglets (4 each for groups A and B and 4 untreated controls), were monitored for postoperative feeding behavior, malaise, evidence for contamination with pathogenic bacteria, and weight gain. All surgical animals were free from incidental contamination from pathogens and environmental organisms with atypical colony types for 35 d. Two piglets in group B died postoperatively (1 during the preliminary experiment and 1 during the final study). Control (group C) piglets gained significantly more weight than did those in group A. These studies demonstrated that surgical resection of the ileal Peyer patches under germfree conditions can be accomplished successfully without compromising piglet health or introducing pathogens and with only a modest reduction in weight gain.

Abbreviation: IPP, ileal Peyer patches.

Intestinal resections are necessary in various clinical conditions, including intestinal obstruction, ulcers, necrotizing enterocolitis, midgut volvulus, malrotation, mesenteric infarction, cancer, trauma, Crohn disease, and Hirschsprung disease.[9,10,18,26,30] In addition, these surgeries may be useful experimental procedures in the study of nutrition, intestinal pathology, and immunity. Resections can be performed by using an open surgical approach or laparoscopically, and the bowel resection procedure depends on the type of intestinal section to be removed.[19] Prognosis is usually good but depends on the patient's age, nutritional status, and general health condition.[20] Some of the risks for this type of surgery include intestinal leakage, short bowel syndrome, damage to nearby organs, wound infection, wound dehiscence, intestinal hernia, perforation of the intestine, and intestinal blockage due to formation of scar tissue.[19]

The ileum is the terminal segment of the small intestine, extending from the end of the jejunum to the ileocecal junction. Although the entire length of the intestinal tract contains lymphoid tissue, only the terminal ileum in swine and other artiodactyls has continuous Peyer patches, the so-called ileal Peyer patches (IPP).[4,8,21] These structures are thought to be a major site of antibody repertoire diversification.[3,21,27] The concept that the IPP play an essential role in B cell development grew out of a proposal that the IPP of neonatal lambs played a similar role as the bursa of Fabricius in chickens.[25] The IPP of sheep are dominated by B cells that undergo rapid negative selection, and the organ appears to involute several weeks after birth.[11,16,24] These observations were followed by other studies indicating that antibody repertoire diversification occurred in the IPP, the diversification was by somatic hypermutation, and that the process was antigen- independent.[22,23] However, these observations and conclusions were never tested through surgical removal of the IPP of lambs or swine. One group of investigators resected the IPP of conventional young pigs but did not measure the effect of their removal on B cell development.[28]

We have used the isolator piglet model[5,6] to demonstrate the need for gut colonization for development of adaptive immunity.[4,7] Testing the prevailing hypothesis regarding the role of IPP in antibody diversification can be accomplished by surgical resection of the ileum of newborns. This testing is best performed under germfree conditions, with the manipulated animals maintained in the controlled environment provided by the isolator system. Another required feature of our assessment includes colonization with a well-defined gut flora.

The work reported here is the first of a series to establish the role of IPP in B-cell development. In this work, surgical intervention of germfree newborn piglets was used with the established technology of the isolator piglet model. The main goal of the current experiment was to establish that we could remove at least 90% (approximately 60 cm) of the IPP from newborn piglets under germfree conditions and then maintain the piglets gnotobiotically. Specifically, we wanted to optimize and evaluate surgical procedures; determine morbidity and mortality rates, differences in weight gain, the ability to be maintained in gnotobiotic conditions; and to identify any effects on clinical parameters resulting from such manipulation. Evaluating effects on clinical parameters was considered important because resection of the IPP under germfree conditions had not been done previously in any species. Described herein are the results of efforts to establish a model that can be used for immunologic research and other applications.

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Materials and Methods

Experimental animals. The experimental protocol used in this study was approved by the IACUC of the South Dakota State University. A total of 17 piglets (5 in the preliminary experiment; 12 in the final experiment) were derived by closed hysterotomy from 2 commercial crossbred (Yorkshire × Landrace) sows on gestation day 112.[17] In summary, a closed hysterotomy was performed by attaching a sterile surgical bubble, which was attached to a sterile isolator unit, to the flank of the anesthetized sow by using an adhesive. The uterus was exposed through an incision made from within the sterile surgical bubble, thus the term 'closed hysterotomy.' Each piglet was removed from the uterus by palpating to locate the piglet, exposing that section of the uterus through the incision site, and making a small incision through the uterus to remove the piglet. After removal from the sow, each piglet was passed through the transfer port from the surgical bubble into the attached isolator.[17] Piglets were reared in custom-made sterile stainless steel isolator units (120 cm long × 60 cm wide × 60 cm high) until at least 35 d old. Stainless steel units were capable of division into four 30 × 60 cm^2 pens or two 60 × 60 cm^2 pens, adjusted according to animal size and space requirements. The steel units were sealed completely from the outside environment by a plastic bubble (120 cm long × 60 cm wide × 60 cm high) with one entry port and a filtered air-exchange system (Figure 1 A). Piglets were trained to drink on their own as early as 2 h after birth by immersing their snouts a few times in their milk bowls. Piglets were fed increasing amounts of milk replacer (Esbilac, Pet Ag, Hamilton IA) 3 times daily, starting at 50 mL on the day of birth, according to the departmental feeding protocol for gnotobiotic piglets. Piglets destined for surgery were fasted from milk replacer (but not water) for 12 h prior to surgery. Piglets in the final study were divided randomly into 3 groups: group A (n = 4) underwent resection of approximately 60 cm of the ileum; group B (n = 4) underwent transection of the ileum 5 cm proximal to the ileocecal junction (sham controls); group C (n = 4) served as nonsurgical controls (Table 1).

Surgical procedure. Sterile instruments and aseptic techniques were used, and piglets were maintained under germfree conditions at all times. Surgery on the germfree piglets was performed inside an incubator (Ohio Care Plus Incubator, OHMEDA Medical, Madison, WI) attached to a sterile isolator unit (Figure 1 B).

Germ-free 2-d-old piglets were placed under general anesthesia with 5% isoflurane (Baxter Pharmaceutical Products, Deerfield, IN) in combination with 95% O_2 for 5 min for induction of anesthesia. Anesthesia was maintained by ventilation with 1% to 2% isoflurane and O_2. Piglets were placed in right lateral recumbency, and a laparotomy was done through a 1.0- to 1.5-in. left flank incision.

In group A (Table 1), ileal length was measured from the ileocecal junction by using marked sterile dental floss placed along the antimesenteric border of the gently stretched ileum. Resection was performed approximately 5 cm proximal to the ileocecal junction and at the tip of the continuous IPP (approximately 60 cm proximal to the ileocecal junction). In sham controls (group B; Table 1), transection was performed approximately 5 cm proximal to the ileocecal junction. Bowel continuity was accomplished by an end-to-end, single-layer, jejunoileal anastomosis with interrupted 5-0 sutures (PDS, Ethicon, Sommerville, NJ). The small intestine and abdominal cavity were flushed with lactated Ringer solution (Hospira, Lake Forest, IL) periodically throughout the surgical procedure. The abdominal wall was closed with interrupted 5-0 sutures (PDS, Ethicon) in the peritoneum and interrupted 3-0 sutures (Monocryl, Ethicon) in the muscle and skin.

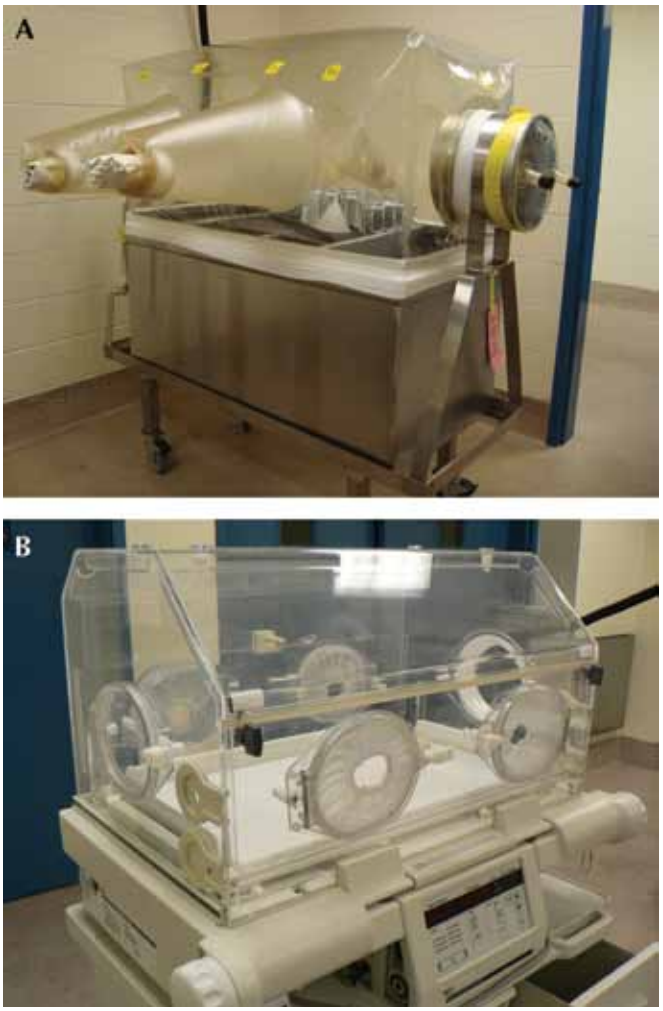

Figure 1. Housing. (A) Stainless steel gnotobiotic isolator, showing a single entry port with an inner and outer cover. All materials brought into the isolator are fogged twice with peracetic acid at 20-min intervals. Experimental animals are handled from the outside by using the gloves attached to the plastic bubble. A filtered-air–exchange system located at the other end of the isolator is used to maintain the gnotobiotic condition of the piglets (B). Front view of the incubator where gnotobiotic piglet surgery was performed. The incubator was attached to the gnotobiotic isolator by using a transfer sleeve attached to the isolator port and the side port of the incubator. Arm holes located at the front and back of the incubator served as an opening to allow the surgeons to insert their hands and forearms to perform the surgery. Temperature inside the incubator was maintained at 37 °C throughout the surgery.

Postoperative management. Piglets were monitored for activity level throughout the recovery period to ensure appropriate recovery from surgery. Piglets received an intramuscular analgesic, buprenorphine hydrochloride (0.01 mg/kg body weight; Henry Schein, Melville, NY), immediately after surgery but prior to recovery from anesthesia and at subsequent 12-h intervals for the first 48 h after surgery. Pigs were given oral lactated Ringer solution after recovery from surgery and resumed feeding with milk replacer at 4 h after surgery. During the course of the experiment, piglets were housed in sterile isolators in a room with an average temperature of 33 °C. Piglets were fed milk replacer (Esbilac, Pet Ag), with the amount adjusted daily to meet their daily nutrient requirements and to maintain adequate caloric intake. Piglets were weighed before

Table 1. Body weights (mean ± 1 SD) on day of surgery (initial; age, 2 d) and necropsy (final; age, 35 d), weight gain, and percentage of weight gain of gnotobiotic piglets in the final study

	Resection (group A; $n = 4$)	Transection (group B; $n = 3$)	Controls (group C; $n = 4$)
Initial weight (kg)	1.01 ± 0.12	1.13 ± 0	0.91 ± 0
Final weight (kg)	7.04 ± 0.42	7.31 ± 0.14	7.71 ± 0.19
Weight gain (kg)	6.03 ± 0.52[a]	6.18 ± 0.14[a,b]	6.80 ± 0.19[b]
Weight gain (%)	595	547	748

Values with different superscript letters were significantly ($P < 0.05$) different from each other.

surgery and before necropsy. Rectal cultures were obtained periodically to assess for contamination prior to colonization with a defined gut flora; any changes in flora after colonization would suggest environmental or pathogenic contamination. Specimens were cultured on blood agar and incubated aerobically and anaerobically at 37 °C for 24 to 48 h. Examination for change in flora included assessment for hemolytic bacteria (not present in the flora used for colonization) and for any unusual morphology of colony forms.

In the preliminary study, 4 germ-free piglets were kept in isolators until 35 d of age. Thereafter, piglets were colonized with a defined gut flora and transferred to a clean room, where they were weaned to a commercial pelleted diet. Necropsy was performed at 70 d of age. The outcome of this experiment formed the basis for the final study, in which piglets were colonized with gut flora 5 d after surgery and maintained until day 35, when they were euthanized for necropsy. Samples were collected from a broad spectrum of tissues for flow cytometry, measurement of C-reactive protein levels in blood samples, measurement of immunoglobulin levels in serum and secretions, repertoire diversification, histochemistry, and immunohistochemistry. Results of the immunologic analyses will be reported elsewhere.

Colonization. Intestinal colonization was done using 3 mL of a defined porcine-derived continuous-flow culture of commensal bacteria (RPCF probiotic flora; Roger Harvey, US Department of Agriculture, College Station, TX).[13,14] The culture contained at least 7 of the following species but was not limited to these organisms: *Enterococcus faecalis*, *Streptococcus bovis*, *Clostridium clostridiforme*, *C. symbiosurn*, *C. ramosum*, *Bacteroides fragilis*, *B. distasonis*, *B. vulgatus*, *B. uniformis*, and *B. caccae*.

Statistical analysis. Weight gain data were calculated according to initial and prenecropsy weights of each piglet. Statistical analysis was conducted by using SAS statistical software (SAS Institute, Cary, NC). ANOVA and the Scheffe test were used to determine statistically significant differences, defined as a P value of less than 0.05.

Results

The preliminary study involved 4 surgical piglets [2 each undergoing resection (group A) or transection (group B)] and 1 unmanipulated control piglet. Three piglets survived surgery (2 in group A and 1 in group B). These piglets were maintained for 5 wk thereafter, during which time they were colonized with defined gut flora. The death of the remaining group B pig was related to postsurgical wound dehiscence.

In the subsequent study, all 4 pigs in group A survived for the entire experimental period, whereas 1 of the 4 piglets in group B died 3 d after surgery (Table 1). At necropsy, the cause of death could not be established. Rectal swabs obtained before or after the surgery showed no bacterial growth. After colonization with defined gut flora, rectal swab cultures produced small nonhemolytic colonies, indicating they were not due to contamination with pathogenic bacteria. No unusual colony types were observed.

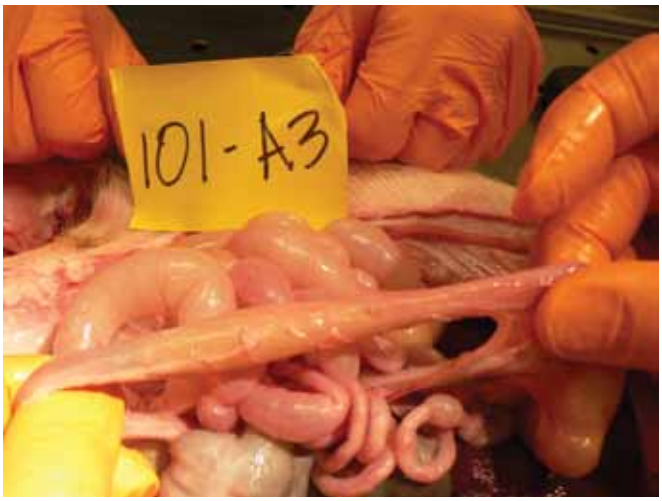

Figure 2. Photograph (obtained at necropsy) of the anastomosis site of a group A piglet in which approximately 60 cm of the ileum was resected. A few adhesions of the small intestine to the abdominal cavity were present, and an apparent postsurgical intestinal regeneration in the terminal ileum adjacent to the resection was noted.

Due to the limited number of animals in the preliminary study, weight data were not analyzed. In the subsequent study, the initial weight of the piglets did not differ significantly between groups (Table 1). However, at necropsy, control piglets (group C) had a significantly ($P < 0.05$) greater weight gain than did resected piglets (group A; Table 1). The general condition of surviving surgical piglets and their feeding habits did not appear to change postoperatively, as all of these animals finished their allotted milk rations within their specified feeding times, were ambulatory immediately after anesthesia recovery, and defecated normally within 24 h after surgery. Blood samples collected 1 wk after surgery lacked detectable C reactive protein, indicating that any inflammation resulting from surgery had been resolved.

At necropsy, the gross anatomic features of the intestinal organs did not differ markedly among pigs in either the preliminary or final study. Piglets in group A showed slight adhesion of the small intestine to the abdominal cavity. In addition, we noted apparent postsurgical intestinal regeneration in the terminal ileum adjacent to the resection (Figure 2).

Discussion

We successfully performed ileal resection and anastomosis through a flank incision approach in 6 germfree piglets. Despite their young age and the challenge of major surgery, we had no mortality in the neonatal gnotobiotic piglets after extensive ileal resection and anastomosis; however, 2 of the 6 piglets with ileal transections (group B) died postoperatively. In addition, we were able to maintain a total of 15 experimental piglets under gnotobiotic conditions until 5 wk of age. Furthermore, 4 piglets in the preliminary study were maintained beyond 5 wk (up to 70 d) and were housed in a semiconventional setting. The mortality of the 2 piglets in the transected group (group B) may be attributed to increased intestinal pressure after surgery,

potentially leading to wound dehiscence and undetermined postsurgical complications.

The survival of humans and animals after small intestinal resection depends on the degree and site or resection and the condition of the remaining gut and its capacity for regeneration.[20] Mice, piglets, and rats survive resection of 50%, 75%, and 90% of the small intestine, respectively, provided that sufficient ileal tissue remains.[1,12,29] One group developed a short-bowel syndrome model by using 7-d-old pigs that received either small-bowel transection or a 75% resection.[15] The small intestine was resected to leave equal lengths of 50-cm residual jejunum and ileum; the resected group had an 8% mortality rate. In addition, 75% small-bowel resection led to clinical short-bowel syndrome, demonstrated by reduced weight gain and typical changes in bowel adaptation parameters.[15] In another study,[2] 80% proximal jejunoileal resection was established in neonatal (older than 48 h) piglets to assess the effect of total parenteral nutrition supplemented with short-chain fatty acids on structural aspects of intestinal adaptation. Although the mortality rate was not indicated, daily weight gain and organ weights did not differ among groups or at the time of euthanasia.[2]

The current study demonstrates that performing ileal resection and anastomosis in neonatal piglets under germfree conditions is feasible and that postoperative outcomes are favorable, at least when piglets are reared gnotobiotically. The studies we report here have paved the way for experiments using isolator piglets lacking IPP to test whether the IPP of artiodactyls are essential for B cell development.[22,23,25] The success of the procedure we have described suggests that the model can also be used for future studies on intestinal microbiology, metabolism, and nutrition.

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