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Alternate Routes of Invasion May Affect Pathogenesis of *Salmonella typhimurium* in Swine

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Transmission of *Salmonella typhimurium* in swine is traditionally believed to occur by the fecal-oral route, with invasion through the intestinal wall and Peyer's patches. However, involvement of the upper respiratory tract may be equally important. An esophagotomy was performed on 6- to 8-week-old pigs. Esophagotomized pigs were challenged intranasally with 10^9 CFU of *S. typhimurium* cells and necropsied at 3, 6, 12, and 18 h postinoculation (p.i.). By 3 h p.i., *S. typhimurium* was recovered from cecum, colon, head, and thoracic tissues and from the middle ileum involving a large number of Peyer's patches. The ileocolic lymph nodes and ileocolic junction were not positive for *S. typhimurium* until 6 and 12 h p.i., respectively. Additional pigs were inoculated transthoracically with 10^9 CFU of *S. typhimurium* and necropsied at 3 and 18 h p.i. By 3 h p.i., all tissues were positive for *S. typhimurium*. Tonsil explants seeded with 10^9 CFU of *S. typhimurium* indicated that within 6 h p.i., *S. typhimurium* was located within the tonsilar crypts. These data show that after intranasal inoculation, *S. typhimurium* rapidly appears in the gut tissues and suggest that the tonsils and lung may be important sites for invasion and dissemination of *Salmonella* species.

Salmonella spp. are ubiquitous in nature, and they have been recovered from nearly all vertebrates (47). Swine, cattle, and poultry are known carriers of *Salmonella* spp. (2, 29), and *Salmonella* spp. in swine are responsible for millions of dollars in lost revenue (40, 49). *Salmonella* spp. have been associated with food-borne illness in human beings (2). Human infection with *Salmonella* spp. typically occurs through ingestion of contaminated food and food products, resulting in severe gastroenteritis. Both short- and long-term carriage in humans has been demonstrated (6, 27).

The pathogenesis of *Salmonella* infections is complex, and many different virulence factors have been identified (5, 12, 14, 16, 23, 28, 31). Although invasion of the intestinal epithelium is thought to be a required virulence attribute of all salmonellae (15, 17, 18, 20, 44), invasiveness may be limited to certain times and host sites during pathogenesis (32).

Experimental infection with *Salmonella* spp. is induced by use of challenge models most often involving inoculation per os, intranasal instillation, and intraperitoneal injection. Use of these different routes results in different degrees of virulence (7, 9, 17, 34, 35, 50) and also affects the carrier status in animals (45, 46). *Salmonella* spp. have also been transmitted following exposure to aerosols or dust (7, 9, 10, 24, 52), and deJong and Ekdahl (11) have suggested that hematogenous and lymphatogenous routes of infection are important in the dissemination of *S. typhimurium*. For our studies, pigs were either esophagotomized and then manually restrained and challenged with *S. typhimurium* intranasally (i.n.) or were anesthetized and challenged by transthoracic inoculation. Tissues were collected over an 18-h time period to determine if they were positive for *Salmonella* species. Additionally, tonsil explants were made from other pigs and were seeded with *S. typhimurium* to determine if *Salmonella* spp. cross the epithelium and, if so, to identify the cells within the tonsil in which they reside.

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MATERIALS AND METHODS

Pigs and experimental design. Twenty-five crossbred pigs were weaned at 10 to 14 days of age and were raised in isolation facilities at the National Animal Disease Center. The pigs were challenged at 6 weeks of age. The esophagotomy experiment was conducted in two separate trials. For trial 1, 3 pigs were challenged and then necropsied at 18 h. One additional pig served as the control. For trial 2, 12 pigs were challenged, and 4 pigs were necropsied each at 3, 6, and 12 h. Two additional pigs served as controls and were necropsied at 3 and 12 h. For the transthoracic experiment, 6 pigs were challenged, and 3 pigs were necropsied each at 3 and 18 h. One additional pig served as the control and was necropsied at 18 h.

Bacteria. *S. typhimurium* χ4232 was used for all experiments and was kindly provided by Roy Curtiss and Sandra Kelly, Washington University, St. Louis, Mo. *S. typhimurium* χ4232 is a derivative of strain 798 (56) and contains a nalidixic acid resistance marker. Prior to use in these experiments, strain χ4232 was used to infect a pig, the strain was recovered from the intestine, and stock cultures were made and maintained at −70°C in glycerol. The frozen stock culture was used to inoculate Luria-Bertani (LB) broth, and the culture was incubated overnight statically at 37°C. The overnight LB culture was used to inoculate (1%) fresh LB broth, and the culture was grown to log phase at 250 rpm at 37°C (optical density at 600 nm, ~0.800). The culture was harvested by centrifugation, the supernatant was discarded, and the pellet was resuspended in a 1/2 total volume of phosphate-buffered saline (PBS) (0.1 M, pH 7.2). The culture optical density was adjusted to ~1.60 for an average final count of approximately 5.0×10^9 CFU/ml.

Esophagotomy surgical procedure. All procedures were approved by the National Animal Disease Center Institutional Animal Care and Use Committee. The pigs were anesthetized with halothane and their esophaguses were exteriorized and cut near the larynx. The caudal end was ligated and returned to the neck area. The cranial end was brought to the outside of each animal and sutured to the skin for drainage. Fluids (Lactated Ringers Solution) were given by subcutaneous injection during and immediately after surgery. Pigs were given 0.1 mg of butorphenol tartrate (Torbugesic) per kg of body weight immediately after surgery and then every 4 h to alleviate pain. The pigs were allowed to recover 1 h prior to challenge.

Challenge protocols. (i) Esophagotomy. For experiments 1 and 2, the pigs were challenged by i.n. inoculation with 1.0 ml of *S. typhimurium* cells (5×10^9 CFU/ml). The pigs were held upright, and approximately 0.5 ml was instilled by drop into each nostril; inoculation was alternated between nostrils during instillation. Control pigs were given PBS containing 1% trypan blue by i.n. inoculation 0.5 h prior to necropsy. Trypan blue was used to estimate initial dissemination of the challenge dose. The pigs were given access to water ad libitum but were restricted from feed.

TABLE 1. Distribution of *S. typhimurium* in tissues of esophagotomized pigs inoculated i.n.

Tissue (n = 4)	No. of positive samples at the following necropsy h:			
	3	6	12	18[a]
Turbinate	4	4	4	3
Tonsil	4	4	4	3
Mandibular lymph node	3	4	4	3
Thymus	3	3	3	3
Trachea	4	4	4	ND[b]
Lung	4	3	4	3
Bronchiole lymph node	4	1	4	3
Spleen	4	2	3	1
Liver	2	1	2	2
Middle ileum	3	1	1	1
ICJ	0	0	2	3
Ileocolic lymph node	0	1	0	1
Cecum	3	3	4	3
Cecal contents	0	1	2	1
Colon	2	1	2	2
Blood	0	0	1	0

[a] n = 3.
[b] ND, not done.

(ii) Transthoracic experiment. The pigs were anesthetized with a mixture of xylazine (4 mg/kg) and ketamine (8 mg/kg). One milliliter of *S. typhimurium* cells (5×10^9 CFU/ml) was introduced into the right caudal lung lobe with a 3-ml syringe attached to a 1.5-in. (1 in. is 2.54 cm) 22-gauge needle. Prior to inoculation, a needle attached to an empty 3-ml syringe was introduced and the barrel was pulled back to aspirate air and to ensure that blood was not present. The barrel of the syringe was changed, the syringe containing the inoculum was attached, and the inoculum was introduced. Pigs recovered from anesthesia within 20 min and were given access to feed and water ad libitum.

Clinical signs and sample collection. Clinical signs, including rectal temperature, were monitored following challenge. Tonsil, nasal, and rectal swabs and swabs from the exteriorized portion of the esophagus were collected and cultured for *Salmonella* species as previously described (56), except that 50 μg of nalidixic acid per ml was added to all of the culture media.

For the esophagotomized pigs, 16 tissues (Table 1) were collected for qualitative bacteriologic culture as previously described (56). Two investigators participated in the necropsy; one removed tissues from the thoracic and head areas while the other removed tissues from the gut area to avoid cross-contamination between areas. Tissues were removed aseptically by using separate sterile instruments and gloves for each tissue. The section from the middle ileum was taken 1 m cranial to the ileocolic junction (ICJ). Seventeen tissue samples (see Table 3) were collected for qualitative culture from the transthoracically inoculated pigs. Quantitative bacteriological studies of select tissues (tonsil, lung, ICJ, and ileocolic lymph node [ICLN] tissues and cecal contents) were also conducted as previously described (57). Tissues were fixed in 10% buffered formalin or glutaraldehyde for histopathology and were processed within 24 h.

Tonsil explants. Tonsil explants were made according to a modification of a method described for mammary explants (48). Briefly, palatine tonsils were aseptically removed from 6- to 8-week-old pigs and were immediately transported to the laboratory, and the fascia was trimmed. The tonsils were washed in Eagle's minimal essential medium (MEM), placed into small beakers containing MEM (the ratio of MEM to the beaker size was 1:2), and allowed to equilibrate for 1 h at 37°C under 5% CO_2. For one experiment, the tonsils were left as whole pieces. For two other experiments, the tonsils were split bilaterally into two pieces, and one half served as the uninoculated control and was placed into a separate beaker. The tonsils were placed in a sterile beaker with medium, and 1 ml of *S. typhimurium* (5×10^9 CFU/ml) in PBS was added to the surface. The beaker was gently swirled. Both the uninoculated control and inoculated tonsils were returned to the incubator. For all experiments, the beakers were covered with sterile aluminum foil and remained stationary. Duplicate sections of the inoculated and uninoculated tonsils were collected at approximately 2, 6, and 21 h p.i. Tissues were washed vigorously in three changes of MEM and once with PBS to remove nonadherent bacteria prior to processing one piece for quantitative bacteriology (57) and one piece for immunohistochemistry. Tonsillar homogenate from uninoculated control tonsils was plated onto Trypticase soy agar plates. Tonsillar homogenate from *S. typhimurium*-inoculated tonsils was plated onto brilliant green agar with sulfadiazine. Bacterial counts are expressed as the means of two (uninoculated controls) or three (inoculated tonsils) trials.

Antiserum to *S. typhimurium*. Four- to five-pound (1 lb = 453.59 g) New Zealand White rabbits were injected with 1 ml of UV-inactivated *S. typhimurium* cells (10^9 CFU/ml) in RIBI adjuvant (RIBI Immunochem Research, Hamilton, Mont.) in multiple sites. A booster injection was given after 4 weeks. Serum samples were collected approximately 2 weeks after the booster injection and were stored at −20°C until use.

Immunohistochemistry. Tonsil explant samples fixed in 10% neutral buffered formalin or Histochoice (Amresco, Solon, Ohio) were embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin and Lillie's Gram stain with a Twort's counterstain (43) to visualize the presence of gram-negative and gram-positive bacteria within the tissues. Serial histologic sections of the tissues were used for immunohistochemical staining with strepavidin biotin alkaline phosphatase (Supersensitive Kit; BioGenex, San Ramon, Calif.). The sections were incubated separately with rabbit polyclonal antisera to *Salmonella* spp. diluted 1:5,000. Positive control tissues were produced by injecting muscle tissue with pure cultures of *S. typhimurium*. Rabbit serum without antibody to *S. typhimurium* was used as the first antibody for negative controls. For electron microscopic evaluation, tissues were fixed as described elsewhere (33). Rabbit antiserum was diluted 1:10 for use in protein A-colloidal gold labelling (30).

RESULTS

Esophagotomy. All body temperatures were within the normal range for all challenge pigs, and no clinical signs were observed through 18 h p.i. At 18 h p.i., some coughing was noted and a clear exudate was present on the nostrils of each pig. Serosanguinous drainage was also evident from the cranial end of the esophagus. Initial drainage was attributed to a small loss of inoculum (<200 μl); consumption of water most likely contributed to later drainage.

S. typhimurium was isolated from all tonsil, nasal, and rectal swabs from pigs necropsied at 3, 6, and 12 h. All tonsil and nasal swabs were *Salmonella* positive for the pigs necropsied at 18 h; however, only one of three rectal swabs was positive. The swabs from the exteriorized portion of the esophagus were positive for all pigs. No fecal samples were available for culture from any of the pigs.

Gross observations at necropsy at 12 and 18 h indicated that the tracheobronchial lymph nodes were hyperemic. Histologically, one pig each at 12 and 18 h p.i. had acute suppurative bronchopneumonia. All other pigs lacked pulmonary suppurative infiltrates. All pigs had minimal to mild interstitial infiltrates of lymphocytes. This change was considered incidental.

Qualitative and quantitative results are described in Tables 1 and 2, respectively. As early as 3 h p.i., *S. typhimurium* was recovered from tissues in the gut including the middle ileum, cecum, and colon. By 18 h p.i., *S. typhimurium* was recovered from all of the tissues for at least one pig. *S. typhimurium* was recovered from the blood of one pig only at 12 h p.i. Although we were able to recover *S. typhimurium* from the gut tissues, we were able to quantitate only one sample from the ICLN of one pig at 6 h.

An estimate of the dissemination of the challenge dose was monitored visually by use of trypan blue. Collection of fluid from the exteriorized portion of the esophagus indicated that

TABLE 2. Average numbers of *S. typhimurium* per tissue from esophagotomized pigs

Necropsy h	Bacterial counts ($\log_{10}$ CFU/g)				
	Tonsil	Lung	ICJ	ICLN	Cecal contents
3	4.69	3.45	N[a]	N	N
6	3.12	−0.10	N	−0.60	P[b]
12	5.00	5.95	P[c]	N	P[c]
18	5.69	5.29	P[d]	P[b]	P[b]

[a] Unable to quantitate; no tissues were positive by qualitative bacteriology.
[b] Unable to quantitate; one tissue was positive from undiluted samples as determined by qualitative bacteriology.
[c] Unable to quantitate; two tissues were positive from undiluted samples as determined by qualitative bacteriology.
[d] Unable to quantitate; three tissues were positive from undiluted samples as determined by qualitative bacteriology.

TABLE 3. Distribution of *S. typhimurium* in tissues of pigs challenged transthoracically

Tissue (n = 3)	No. of positive samples at the following necropsy h:	
	3	18
Turbinate	3	3
Tonsil	3	3
Mandibular lymph node	3	3
Thymus	3	3
Esophagus	3	3
Trachea	3	3
Lung	3	3
Bronchiole lymph node	3	3
Spleen	3	3
Liver	3	3
Middle Ileum	3	3
ICJ	3	3
Ileocolic lymph node	3	3
Cecum	3	3
Cecal contents	2	3
Colon	3	3
Blood	3	3

approximately one-fifth of the challenge dose was lost through this opening. Blue dye was observed in the turbinate, tonsils, trachea, and upper branches of the bronchus within 0.5 h p.i., the time between challenge and necropsy.

Transthoracic experiment. A decrease of 1.1°C in rectal temperatures was observed for pigs necropsied at 3 h p.i., and all pigs experienced mild dyspnea. Temperatures for the pigs necropsied at 18 h decreased by 1.4°C from the baseline and then rose 1.0°C above the baseline by 18 h p.i. At 6 h p.i., pigs were shivering and lethargic. By 18 h p.i., all pigs were lethargic and inappetent, and moderate dyspnea was observed.

All tonsil, nasal, and rectal swabs were positive at 3 and 18 h p.i. Composite fecal samples from the pigs necropsied at 18 h were positive at 6 and 18 h; no sample was available at 3 h. Quantitative bacteriological study of the fecal samples was not done.

At necropsy, gross observation included petechial hemorrhages in all thymus tissue from the pigs necropsied at 3 h, diffuse hemorrhage in the right lung lobes at 18 h, and enlargement of the tracheobronchial lymph nodes and ICLNs. Histologically, two pigs at 18 h had acute suppurative infiltrates. The remaining pigs lacked suppurative infiltrates but had minimal to mild interstitial infiltrates of lymphocytes; this change was considered incidental.

Qualitative and quantitative results are presented in Tables 3 and 4. By 3 h p.i., all tissues from all pigs, with the exception of the cecal contents for 1 pig, were positive for *S. typhimurium*. All tissues from all pigs were positive at 18 h. Quantitative counts were highest in the lung tissues for both necropsy times. Counts increased for all other tissues between 3 and 18 h p.i.

TABLE 4. Average numbers of *S. typhimurium* per tissue from pigs challenged transthoracically

Necropsy h	Tissue ($\log_{10}$ CFU/g)				
	Tonsil	Lung	Ileum	ICLN	Cecal contents
3	3.35	6.51	4.19	1.65	P[a]
18	5.19	7.24	5.61	4.06	5.57

[a] Unable to quantitate; two tissues were positive from undiluted samples as determined by qualitative bacteriology.

Control animals. Control animals were culture negative for *S. typhimurium.*

Tonsil explants. Average bacterial counts for non-*Salmonella* spp. were 5.95 $\log_{10}$ at 0 h. By 2 h p.i., bacterial counts had increased to 7.04 $\log_{10}$. Counts were 7.70 $\log_{10}$ and 7.56 $\log_{10}$ for 6 and 21 h p.i., respectively. For the *S. typhimurium*-inoculated tonsils, *Salmonella* bacterial counts were 9.58 $\log_{10}$, 6.83 $\log_{10}$, and 8.04 $\log_{10}$ for 2, 6, and 21 h p.i., respectively.

Microscopic examination of the negative control tonsils at 0 h revealed rare gram-positive cocci, which were immunonegative, within the tonsilar crypts. At 6 h p.i., the small colonies of gram-positive immunonegative coccoid bacteria persisted. Gram-positive rods and rare filamentous bacteria were evident in some sections. The tonsilar tissue was slightly edematous, with expansion of the tissue architecture at 21 h p.i. Large numbers of gram-positive colonies were evident within the crypts and present within the lamina propria in one focus in which the epithelium was separated from the subepithelial tissue. There was no invasion of intact epithelium by bacteria in any control tissue, and none of the bacteria seen by Gram stains were immunopositive with anti-*Salmonella* antibody.

Microscopic examination of the *S. typhimurium*-inoculated tissues at 2 h p.i. did not detect any bacteria with Gram-stained sections. Immunohistochemistry revealed rare immunopositive bacteria on the surface of the tonsilar epithelium above the crypts. Examination at 6 h p.i. revealed rare gram-positive coccoid-shaped and numerous gram-negative rod-shaped bacteria on the surface of the crypt epithelium and within the tonsilar crypts. By immunohistochemistry, numerous gram-negative bacilli were immunoreactive for the *Salmonella* antibody (Fig. 1). At 21 h p.i., large colonies of gram-positive cocci were present within the crypts, and rare cells were evident in the ulcerated epithelial lining. Large colonies of gram-negative rods covering the crypt lumen, within the crypts, and extending through the epithelial lining and into the surrounding diffuse lymphoid tissue were evident. Most of the gram-negative, rod-shaped bacteria were identified as *Salmonella* spp. by immunohistochemistry. Immunopositive bacteria and bacterial particles covering the superficial lumen to the crypt, filling the crypt, and extending through the crypt epithelium were evident. Immunopositive cells were also present within macrophages in the diffuse lymphoid tissue and within the lymphoid nodules of the tonsil in several sections (Fig. 2).

By 6 h, electron microscopic examination of the tonsil revealed gold-labelled gram-negative bacilli on the surface of the epithelium and within the lamina propria. Identification of intracellular vesicles in which *S. typhimurium* was contained was not determined. Divisional septa of gold-labelled bacilli were seen at 21 h, indicating that replication was occurring within the tissue (data not shown). Evidence of cellular degeneration was observed by 21 h.

DISCUSSION

Historically, *Salmonella* species have been described as enteric pathogens, and transmission was thought to occur by fecal-oral exposure. The data presented in this study give evidence for the rapid accumulation of a population of *S. typhimurium* in the gut by a route(s) other than the classic oral route of inoculation. Although these data do not prove that nasal or respiratory exposure results in disease, they confirm previous observations (11) that both lymphatogenous and hematogenous routes are important in the dissemination of *S. typhimurium* and provide evidence that the tonsils and lungs are important sites for invasion by *S. typhimurium*.

There is little doubt that infection with *Salmonella* spp. in

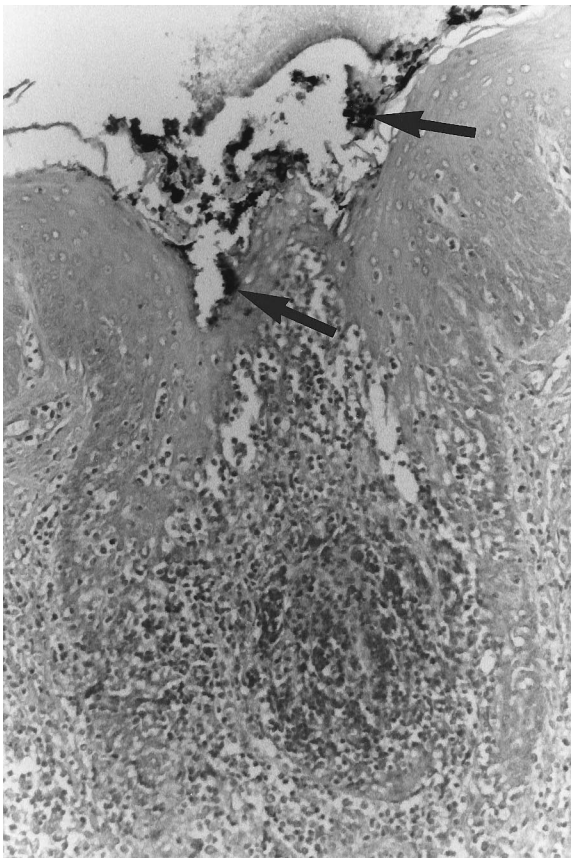

FIG. 1. Immunopositive cells (arrows) on the surface of the crypt and superficial epithelium from a section of tonsil taken at 6 h p.i. Approximate magnification, ×50.

animals can result in a systemic infection (54). Although *S. typhimurium* was recovered from the blood of only one of six esophagotomized pigs, *S. typhimurium* was recovered from the blood of all six pigs inoculated transthoracically. This suggests that involvement of the lung may be more important for the development of septicemia, while the tonsil may be more associated with lymphatogenous dissemination of the organism. Introduction of *S. typhimurium* into the blood during challenge in the transthoracic experiment cannot be ruled out, since a small possibility that the inoculum was introduced into blood exists.

The tissues of the nasal-associated lymphoid tissue (NALT) are the major mucosal inductive site of the upper respiratory tract (4) and include the palatine and nasopharyngeal tonsils, which encounter infectious agents and environmental antigens that are ingested or inhaled. Together, these tissues provide an environment for the induction of immune cells that relocate to distant effector tissues (41). deJong and Ekdahl (11) esophagectomized calves prior to oral challenge with *S. typhimurium* and collected tissues for bacteriologic examination. Our esophagotomy experiments with swine show differences from the results for the calves in the numbers of positive tissues. Most notably, deJong and Ekdahl were unable to recover *S. typhimurium* from the ileum. One difference may be attributed to the route of challenge; they used an oral challenge which may not have involved all of the NALT, while our i.n. challenge should have maximized involvement of the NALT. One other difference may be attributed to sampling sites.

Although the tonsil has been described as one of the primary sites of invasion by bacteria, with subsequent spread to the superpharyngeal lymph nodes, it is unlikely that it is the only portal of entry for bacteria and other pathogens (38). In newborn animals, investigators have demonstrated that bacteria enter via the tonsils, small intestines, lungs (37) and umbilical cords. Pigs spend a considerable amount of time rooting in their environment. During rooting, a pig's nose contacts many surfaces, including the bodies of other pigs, environmental areas, water, and feed. One can usually observe feed and other particles lodged within the nasal cavities. Therefore, the behavior and mode of feeding pigs (and presumably other animals) ensures that pathogens in the food and environment invariably enter the nasal cavity (1). Bacteria ingested with food can lodge within tonsilar crypts and multiply within epithelial keratin and crypt material (1). Replication of *S. typhimurium* within the lamina propria provides evidence for in vitro multiplication within the tonsil, while Tables 2 and 4 provide evidence for in vivo multiplication. It has been suggested that one function of the tonsil may be the development of immunity to bacteria in the environment (37). If extracellular replication is occurring, the population of bacteria continues to increase, and the chance for immune clearance is decreased (8). Infection via the nasal cavity may also prolong the carrier state (45, 46), which may be related to the immune status of the host (22). Interestingly, we (22) and others (56, 57) have demonstrated that the tonsil is a primary site of colonization in pigs that have become subclinical carriers. This suggests that while the presence of low numbers of *S. typhimurium* within the tonsil may provide continual stimulation for expression of an immune response, the immune response may not be sufficient to clear the organism from the host and thus allow for development of a carrier state.

The presence of trypan blue in the trachea following i.n. challenge in esophagotomized pigs indicates that the lungs were inoculated following i.n. inoculation. It is also quite probable that the lungs are inoculated following oral inoculation. Previously, we observed some respiratory distress in swine following oral inoculation with *S. typhimurium* (data not shown). Although we did not recover and culture *S. typhimurium* from alveolar macrophages specifically, we did recover high numbers of *S. typhimurium* from the lungs of pigs from both esophagotomy and transthoracic experiments. Thus, it is difficult to envision that the *S. typhimurium* was not taken up by the alveolar macrophages, which may act as vehicles for disseminating salmonellae. Swine have a large number of macrophages in their lungs (55), and resistance may be attributed to the ability of phagocytic cells to inactivate viable bacteria and prevent further dissemination into the reticuloendothelial organs (39). Our data indicate that there may be impaired ability of the alveolar macrophages to contain *S. typhimurium* within hours after infection. Bacterial levels in the lungs of esophagotomized pigs indicated that the number of CFU per gram fell below 10^3 between 3 and 6 h postchallenge. However, between 6 and 12 h postchallenge, the levels increased from 10^0 to 10^5. Bacterial levels also increased in pigs inoculated transthoracically. These data suggest that initial resistance and killing are eventually overcome, allowing for dissemination of large numbers of *S. typhimurium* within a short time span.

Recovery of *S. typhimurium* from the thymus and the development of petechial hemorrhages in the thymus following challenge suggest that some toxic event, directly or indirectly related to infection with *S. typhimurium*, that may target the development of specific cell populations occurs. We hypothesize that this may have an effect on the development of a protective immune response. The thymus is a primary site for

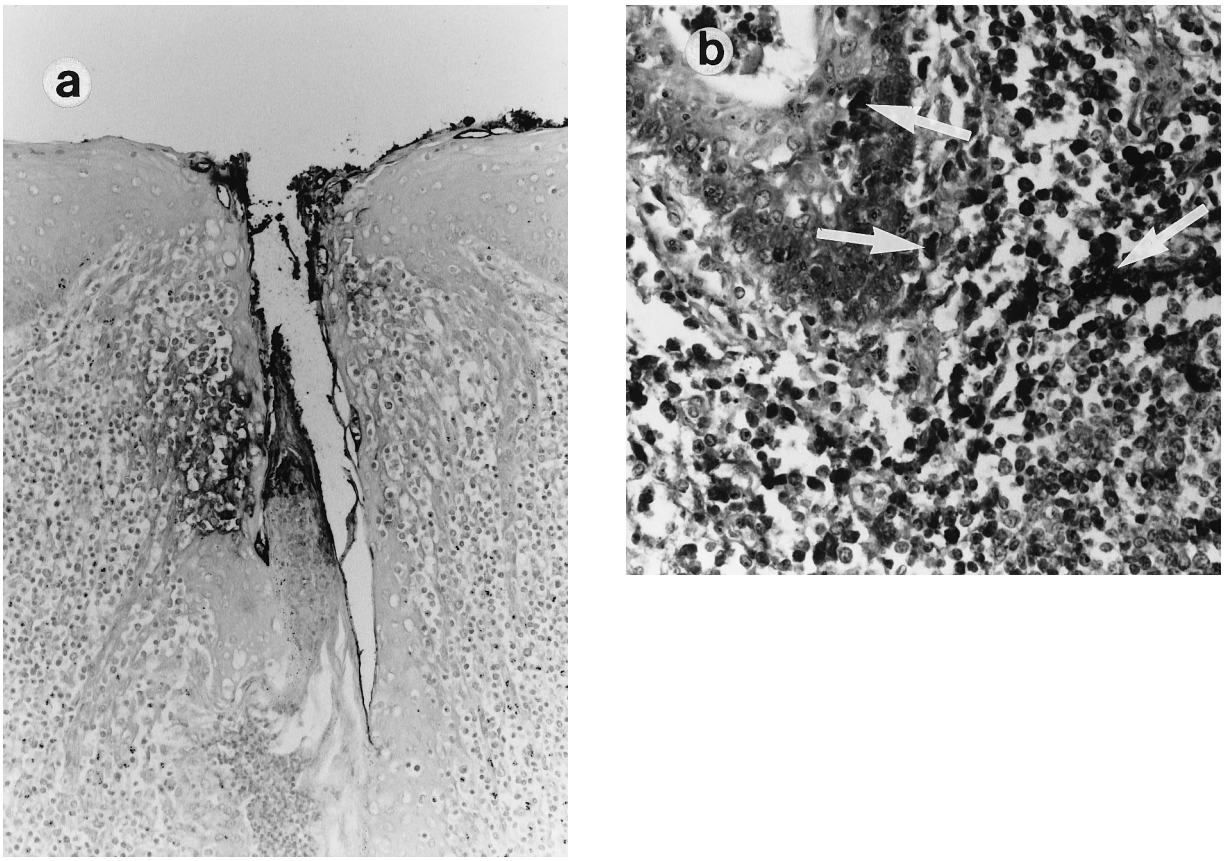

FIG. 2. (a) Immunopositive bacteria and bacterial particles covering the superficial lumen to the crypt, filling the crypt, and extending through the crypt epithelium of a tonsil section taken at 6 h p.i. Approximate magnification, ×50. (b) Immunopositive cells were also observed in the macrophages (arrows). Approximate magnification, ×100.

T-cell development. Impaired T-cell-mediated immunity has been shown to promote translocation of certain indigenous bacteria (36). Thus, T-cell-dependent immunity appears to play a significant role in inhibiting bacterial translocation from the gastrointestinal tract (42). We suggest that if the thymus is impaired and if *Salmonella* species have entered via the oral route, they may be translocated across the gut epithelium. Translocation for other tissues may also be affected. T-cell depletion has been shown to increase *S. typhimurium* translocation to the liver and spleen, but not to the mesenteric lymph nodes (19). However, following i.n. inoculation, the presence of *S. typhimurium* in the ICLNs but not in the ICJ at 6 h (Table 1) indicates that translocation across the intestinal epithelium is not necessary for colonization of the ICLN. Hassan and Curtiss (25) have shown that intra-air sac inoculation with *S. typhimurium* caused temporary atrophy of the thymus and bursa of Fabricius and hypertrophy of the spleen in 4-week-old chickens; partial repopulation of the lymphoid organs was observed within 1 week after infection. It is possible that the repopulated cells may be different from the original population. Further studies are warranted to further define cell populations in the thymus following challenge with *S. typhimurium* and to elucidate their role in translocation.

Investigators (8) have implied that infection of Peyer's patches may be due as much to blood-borne infection as to invasion from the gut. Recovery of *S. typhimurium* from the middle ileum (area associated with Peyer's patches), colon, and cecum prior to recovery from the ICJ, ICLNs, or cecal contents suggests that bacterial translocation in the intestine may not be a prerequisite for virulence. *S. typhimurium* within the intestinal lumen appears to preferentially adhere to and enter the M cells located within the epithelium that covers the Peyer's patches (26). However, macrophages in the Peyer's patches could play a role in trafficking and act as "Trojan horses" in disseminating *Salmonella* throughout the host (51). Tissue macrophages may also facilitate transport to an extraintestinal site without intracellular killing (53). Initially, translocation (3) across the epithelium could not have occurred in the esophagotomized pigs, and we suggest that dissemination of the population of *S. typhimurium* that we observed in the gut tissue is not dependent on M-cell translocation. Interestingly, it appears that translocation occurs in the opposite direction, as is evidenced by recovery of *S. typhimurium* from the cecal contents at 6 h, presumably as a result of translocation from the cecal wall. We have also observed the development of microscopic lesions in the gut in the region of lymphoglandular complexes and within venules which appear to be progressing outward (13).

Since transepithelial transport is essential in the development of the mucosal immune response (21), *Salmonella* spp. that bypass oral introduction into the host may not stimulate an adequate immune response. We hypothesize that if the predominant route of inoculation is via the nasal-respiratory route, the host immune response may be altered or delayed. *S. typhimurium* would traffic via lymphatogenous and hematogenous routes, and numbers would increase in both phagocytic

and nonphagocytic cells. At some point, either in conjunction with an aerosol or a nasal exposure or at some time afterward, there would be some level of oral inoculation via swallowing. However, the population that was ingested may not play a significant role in disease (9), since it was preceded by nasal infection. On the other hand, the oral population that comes in contact with the tonsil and other pharyngeal lymphoid tissue may play a significant role through lymphatic dissemination. The sequence of exposure, i.e., oral-respiratory versus respiratory-oral exposure, may affect the immune response, which may in turn affect the severity of the disease.

Based on these studies, a *Salmonella* population entered the host via the tonsils, NALT, and lungs, bypassing traditional oral-fecal dissemination. This population may in some way, either by virulence attribute or host resistance, affect the outcome of disease, the immune response, and/or the carrier state. Organisms tend to be rapidly eliminated unless an early systemic foothold occurs (8). Further study of the *Salmonella* population that bypasses the oral route is warranted.

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