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Host Specificity of Filamentous, Segmented Microorganisms Adherent to the Small Bowel Epithelium in Mice and Rats

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Germfree rats and mice were given by gavage samples of ileal homogenates prepared from conventional rats and mice. Filamentous, segmented procaryotes adhered to the small bowel epithelium in the ex-germfree mice only when the homogenate was made from mouse bowel and in the ex-germfree rats only when the inoculum came from rats. Thus, the filamentous microorganisms are host animal specific.

Filamentous, segmented microorganisms inhabit the lower small intestine of mice (6), rats (10), and chickens (5, 9). The filaments range from 2 to 80 μm in length and 0.7 to 1.8 μm in diameter and have an intimate relationship with their host animal, being attached by one end to the epithelium of the lower small intestine (2–4, 6, 14). The attaching end of the filament is tapered and inserted into a depression that is formed in the cytoplasmic membrane of the host epithelial cell. In the formation of such attachment sites, the host cell membranes and adjacent cytoplasm may undergo a sol-to-gel conversion stimulated by a substance(s) associated with the filamentous organisms (14).

Reproduction and dispersion of the filaments to allow continuing colonization of the intestinal mucosa may occur by several means: intrasegmental bodies which are released and which attach to epithelial cells, later forming segmented filaments; constriction and subsequent separation of parts of the segmented filaments; or endospore formation within segments (2–4). Although the filamentous, segmented organisms are associated with villous surfaces throughout the lower part of the rodent small intestine, they are particularly numerous in the vicinity of Peyer's patches. The epithelial surface of these accumulations of lymphoid tissue can be densely colonized by the microbes, which attach at the junctions of M cells (1, 8).

Despite numerous attempts, these filamentous, segmented microorganisms have never been cultured in recognizable form in vitro. They are readily detected by light microscopic examination of appropriately stained sections of intestine, however, and by scanning or transmission electron microscopy. The cells have the procaryotic structure of gram-positive bacteria but usually stain gram negatively (3, 4, 10).

Microorganisms indigenous to the gastrointestinal tract, particularly those which colonize epithelial surfaces, may be highly adapted to their habitat and thus for colonizing specific animal hosts (11). We have tested this hypothesis for the filamentous, segmented microbes by assessing whether such organisms present in ileal homogenates prepared from conventional rats and mice will colonize the epithelial surface of the lower small intestine of germfree rats or mice.

Adult germfree Crl:CD-1(ICR)GN mice, males and females, originally obtained from Charles River Breeding Laboratories, Inc., Wilmington, Mass., and then bred in our own colony, were housed on sterile Ab-Sorb-Dri bedding (Ab-Sorb-Dri Inc., Garfield, N.J.) in plastic cages maintained in flexible plastic isolators and were given autoclave-sterilized food (Wayne Lab-Blox, Allied Mills, Chicago, Ill.) and water ad libitum, as described previously (13). Adult germfree Crl:CD(SD)GN rats, males and females, obtained from Charles River Breeding Laboratories, also were housed on Ab-Sorb-Dri in plastic cages in isolators and were given water and sterilized Lab-Blox ad libitum. These germfree animals were anesthetized (nembutal) and given by gavage samples of ileal homogenate prepared either from conventional mice (CD-1) or from conventional rats (Sprague-Dawley). Previous examinations had confirmed that conventional mice and rats housed in our facility harbored filamentous, segmented microorganisms in their small intestine. Homogenates of conventional rat ileum were prepared from lengths (3 to 4 cm) of lower small intestine collected from three rats (males and females). These tissues were homogenized in prereduced, anaerobically sterilized brain heart infusion medium under oxygen-free gas (7). Lengths (2 to 3 cm) of conventional mouse ileum collected from six mice (males and females) were used to prepare mouse homogenates in a similar manner. The homogenates were maintained in a reduced condition under oxygen-free gas (N_2, 80%; CO_2, 10%; H_2, 10%) throughout the experimental procedures. In the case of rat ileal homogenates, germfree mice were inoculated with the material first, followed by inoculation of germfree rats from the same homogenate. Conversely, in the case of mouse ileal homogenates, germfree rats were inoculated first, followed by inoculation of germfree mice from the same homogenized material. Three experiments were performed in this manner. Each experiment differed in that the inoculated animals were examined at a different time interval after inoculation: 4 days, 5 weeks, and 7 weeks. At those times, the animals were killed (carbon dioxide narcosis), and portions of the lower small intestine from each were removed for examination. The specimens were frozen at −20°C in a microtome-cryostat; 4-μm-thick sections were prepared and stained (hematoxylin-eosin; Gram stain) as described previously (15). Stained sections were examined by light microscopy for filamentous, segmented microorganisms.

Such organisms present in the ileal homogenates prepared from conventional rats colonized the ileum of all of the germfree rats inoculated with this material (Table 1). However, they did not colonize the ileum of germfree mice. Conversely, the filaments present in the homogenates prepared from conventional mice colonized the ileum of germfree mice but not that of germfree rats (Table 1). Thus, we conclude that the filamentous, segmented microorganisms exhibit host specificity; the filaments seen in mouse ileum

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TABLE 1. Detection of filamentous, segmented microorganisms in the ileum of ex-germfree rats and mice given samples of ileal homogenates from conventional rats or mice by gavage

Expt	Ileal homogenate[a] from conventional:	Time after inoculation	Filamentous segmented microbes present in ileum of ex-germfree[b]:	
			Mice	Rats
1	Rats	4 days	0/2	2/2
	Mice	4 days	5/5	0/2
2	Rats	5 weeks	0/4	6/6
	Mice	5 weeks	6/6	0/6
3	Rats	7 weeks	0/11	5/5
	Mice	7 weeks	11/11	0/5

[a] For each experiment, the ileal homogenate used was prepared from a different group of rats or mice.

[b] Number of animals harboring filamentous microbes/number of animals examined; observed by light microscopy.

only colonize the mouse gastrointestinal tract, and the filaments seen in rat ileum only colonize rats.

The basis of this host specificity remains speculative. We would propose, however, that the initial contact between filamentous microbe and epithelial cell involves recognition of specific microbial adhesives and mucosal surface receptors (11). These adhesive factors may differ in the mouse and rat intestinal ecosystems. Alternatively, host secretions and other physiological properties could differ between rats and mice, such that conditions in the small intestine are not conducive to colonization of that site by other than highly host-adapted organisms.

Host specificity in colonizing germfree animals has been demonstrated for one other group of gastrointestinal bacteria, the lactobacilli. Certain strains of lactobacilli can colonize squamous epithelial surfaces in the alimentary canals of rodents, chickens, and pigs (11). Strains of lactobacilli isolated from rodents colonize only rodent stomach epithelium, whereas strains isolated from chickens colonize only crop epithelial surfaces. Strains of lactobacilli isolated from mice will colonize the stomach epithelium of rats, however, and vice versa (16). The filamentous, segmented procaryotes exhibit a greater degree of host specificity, distinguishing between rodents of two species. We speculate that such host specificity may be found for other indigenous microorganisms that live in close association with epithelial surfaces in the gastrointestinal tract (11).

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