Citrobacter rodentium

(formerly *C. freundii* biotype 4280 and *Citrobacter* genomospecies 9)

- *C. rodentium* and MPEC (mouse pathogenic *E. coli*) are synonymous (Luperchio 2000)

Host species:

- laboratory mice
- one report about an epidemic outbreak in a gerbil colony (de la Puente-Redondo, 1999)
- one report of an outbreak in a guinea pig colony (Ocholi 1988)

Organotropism:

- etiologic agent of transmissible murine colonic hyperplasia (TMCH)
- large bowel (descending colon is most affected)

Clinical disease:

- infection in most adult mice is subclinical and self-limiting (Barthold 1978)
- suckling mice, adult animals of some inbred strains (Barthold 1977, Itoh 1988, Silverman 1979), Han:NMRI mice (Bieniek 1976) and transgenic lines (Maggio-Price 1998) are more susceptible and demonstrate clinical signs
- clinical signs are nonspecific and include ruffled coat, weight loss, depression, stunting, perianal fecal staining, pasty dark feces and dehydration
- variable incidence of rectal prolapse in mice of all ages is indicative of infection (Brennan 1965, and others)
- mice that recover may be refractory to reinfection (Barthold 1980)
- streptomycin in the drinking water may influence the severity of the disease (necrosis, colitis) (Luperchio 2000)
- age, host genetic background, diet and indigenous microbiota influence disease expression (Luperchio 2001)
- mucosal hyperplasia is more severe in outbred NIH Swiss mice as compared with C3H/HeJ, C57BL/6J and DBA/2J mice (Barthold 1977)
- moderate hyperplasia in C3H/HeJ mice (Barthold 1977)
- least degree of hyperplasia in C57BL/6J and DBA/2J mice (Barthold 1977)
- commercial diets effect the baseline colon morphology and presumably the epithelial cell turnover rate – the dietary constituents responsible for this effect are unknown (Barthold 1977)
- germfree CF1 and C3H mice are highly susceptible, germfree C57BL/6 and NC mice are susceptible, and germfree BALB/c are resistant to infection (Itoh 1988)
- $CD4^{-/-}$ or TCR-$\beta^{-/-}$ mice develop polymicrobial sepsis and end-organ damage (abscesses) and succumb during acute infection (Bry 2004)

Pathology:

- colitis

- hallmark pathologic lesion: colonic hyperplasia with limited inflammation and epithelial cell hyperproliferation in the descending colon (Barthold 1978)
- characterized by crypt elongation, increased mitotic activity, mucosal thickening, variable mucosal inflammation, crypt abscesses, occasional erosions and ulcers, healing and goblet cell hyperplasia (Barthold 1978, National Research Council 1991)
- necrosis of the colonic mucosa and severe colitis most notably in suckling mice (Luperchio 2000)
- grossly thickened and rigid distal colon, devoid of formed feces
- cecum is often empty and contracted
- with increasing severity of disease, the entire colon, and less frequently, the cecum and ileum may be involved
- animals of some inbred strains and transgenic lines develop lesions as severe as those seen in suckling mice: neutrophil infiltration of mucosa and submucosa, mucosal erosions and necrosis (Barthold 1978)
- mucosal hyperplasia is dependent on the host immune response (Higgins 1999)
- infection generates a predominately lymphocytic infiltrate, characterized by $CD4^+$ T cells situated near the proliferative epithelial crypts (Higgins 1999)
- innate immunity can mediate acute responses to infection, but T and/or B lymphocytes mediate most of the tissue pathology and inflammation in the later stages of infection (Vallance 2002)
- bacteremia and extra-intestinal infection are not hallmarks of infection, though recovery of bacteria from blood and liver and spleen has been reported (Luperchio 2001)
- B cell-deficient ($MuMT^{-/-}$) or B and T cell-deficient (recombinase-activating gene $2^{-/-}$) mice develop severe pathology in the colon and internal organs and deteriorate rapidly during acute infection (Bry 2004)
- inflammatory and crypt hyperplastic responses in $RAG1^{-/-}$ mice are transient and infection is often fatal (Vallance 2002)
- $RAG1^{-/-}$ mice respond to infection primarily with a granulocytic infiltration of the colonic mucosa (Vallance 2002)
- hyperplastic responses do not occur in interferon (IFN)-γ receptor-deficient mice (Higgins 1999)
- depletion of IFN-γ prevents crypt hyperplasia (Artis 1999)

Morbidity and mortality:

- little morbidity and mortality in most adult mice, while mortality or runting is seen in weaning-age mice (Barthold 1978, Barthold 1977)
- increased level of mortality accompanied by a high incidence of rectal prolapse in outbred Swiss-Webster mouse (Ediger 1974)
- high mortality in T-cell receptor αβ transgenic mice (Maggio-Price 1998)
- wild-type mice clear the infection; T and/or B lymphocytes are required to clear the infection (Vallance 2002)

- only limited mortality in most inbred and outbred strains
- severity of hyperplasia does not correlate with mortality: C3H/HeJ mice did not develop more severe hyperplasia as compared to outbred Swiss-Webster mice, but C3H/HeJ mice exhibited 45% mortality while no mortality was observed in Swiss-Webster mice (Luperchio 2001)
- C57BL/6 mice depleted of $CD4^+$ T cells are highly susceptible to infection and develop severe colitis (Vallance 2003)
- LPS-hyporesponsive C3H/HeJ mice experience more rapid and extensive bacterial colonization than SCID mice – high bacterial load is associated with accelerated crypt hyperplasia, mucosal ulceration and bleeding, together with very high mortality rates (Vallance 2003)
- immunodeficient mice (mice lacking IL12, IFN-γ, TNF receptor or both T and B lymphocytes) are more susceptible to infection than immunocompetent mice (Goncalves 2001, Simmons 2002, Vallance 2002, Vallance 2003) and infection is often fatal in former mice

Zoonotic potential:

- none

Interference with research:

Physiology

- experimental stress can evoke more severe disease in infected mice
- increase in total cellular β-catenin accompanied by an increase in nuclear β-catenin concentrations; elevated levels preceded crypt elongation (Sellin 2001)
- increased transcription of EGR-1 with subsequent activation of the MEK/extracellular signal-regulated kinases (de Grado 2001)
- increase in production of keratinocyte growth factor, which induces cell proliferation (Higgins 1999; Bajaj-Elliott 1998)

Immunology

- mice with deficiencies in cell-mediated immunity or mice lacking T and B cells are more susceptible to infection with *C. rodentium* (MacDonald 2003)
- colonic hyperproliferation is associated with cytokinetic alterations (Barthold 1979)
- increase of IL-10 secretion and inhibition of IL-2 and IL-4 secretion by mitogen-stimulated murine spleen cells (Malstrom 1998)
- variable effect on IFN-γ secretion, whereas the effect of enteropathogenic Escherichia coli lysates is inhibitory (Malstrom 1998)
- suppression of lymphocyte activation *in vitro* (Klapproth 2000)
- highly polarized Th 1 immune response, characterized by increased levels of IL-12, IFN-γ and TNF-α mRNA (Higgins 1999)
- infected IL-12p40$^{-/-}$ and IFN-γ$^{-/-}$ mice mount anti-*Citrobacter* serum and gut associated IgA responses and strongly express inducible NO synthase (iNOS) in mucosal tissue, despite diminished serum nitrite/nitrate levels (Simmons 2002)

- up-regulated expression of the mouse β-defensins (mBD)-1 and mBD-3 in colonic tissue in C57BL/6 mice; in contrast, only up-regulated expression of mBD-3 in IL-12- and IFN-γ-deficient mice (Simmons 2002)

Oncology

- *C. rodentium*-induced hyperplasia can alter chemical carcinogenesis in the large bowel (Barthold, 1977, 1980)
- hyperplastic state of the colon serves as a promoter for colon tumorigenesis (Barthold 1977)
- transient hyperplastic state increases susceptibility to the carcinogenic effect of 1,2-dimethylhydrazine (DMH) in NIH Swiss mice
- DMH administration concomitant with hyperplasia reduces the latency period for early neoplastic lesions, however hyperplasia has no effect on already established tumors
- hyperplastic lesions may be confused with neoplasia because associated cytokinetic alterations share several common features with those observed in neoplasia (Barthold, 1979; Pullinger 1960)
- increase in cellular concentrations of cyclin D1 and c-Myc (proteins maintaining proliferation status)

References:

Artis, D., C. S. Potten, K. J. Else, F. D. Finkelman, and R. K. Grencis. 1999. Trichuris muris: host intestinal epithelial cell hyperproliferation during chronic infection is regulated by interferon-gamma. Exp. Parasitol. 92: 144-153.

Bajaj-Elliott M., R. Poulsom, S. L. Pender, N. C. Wathen, and T. T. MacDonald. 1998. Interactions between stromal cell-derived keratinocyte growth factor and epithelial transforming growth factor in immune-mediated crypt cell hyperplasia. J. Clin. Investig. 102: 1473-1480.

Baker D. G. 1998. Natural pathogens of laboratory mice, rats, and rabbits and their effects on research. Clin. Microbiol. Rev. 11 (2): 231-266

Barthold, S. W. 1979. Autoradiographic cytokinetics of colonic mucosal hyperplasia in mice. Cancer Res. 39:24-29.

Barthold, S. W. 1980. The microbiology of transmissible murine colonic hyperplasia. Lab. Anim. Sci. 30: 167-173.

Barthold, S. W. and D. Beck. 1980. Modification of early dimethylhydrazine carcinogenesis by colonic mucosal hyperplasia. Cancer Res. 40:4451-4455.

Barthold, S. W., G. L. Coleman, P. N. Bhatt, G. W. Osbaliston and A. M. Jonas. 1976. The etiology of transmissible murine colonic hyperplasia. Lab.Anim. Sci. 26:889-894.

Barthold, S. W., G. L. Coleman, R. O. Jacoby, E. M. Livstone and A. M. Jonas. 1978. Transmissible murine colonic hyperplasia, Vet. Path. 15:223-36.

Barthold, S. W., G. W. Osbaldiston, and A. M. Jonas. 1977. Dietary, bacterial and host genetic interactions in the pathogenesis of transmissible murine colonic hyperplasia. Lab. Anim. Sci. 27: 938-945.

Barthold, S. W., and A. M. Jonas. 1977. Morphogenesis of early 1,2dimethylhydrazine-induced lesion and latent reduction of colonic carcinogenesis in mice by variant of *Citrobacter freundii*. Cancer Res. 37:4352-4360.

Bieniek, H., and B. Tober-Meyer 1976. Zur Ätiologie der Colitis und des Prolapsus recti bei der Maus. Z. Versuchstierk. 18: 337-348.

Brennan, P. C., T. E. Fritz, R. J. Flynn and C. M. Poole. 1965. *Citrobacter freundii* associated with diarrhoea in laboratory mice. Lab. Anim. Care 15: 266-275.

Bry L., and M. B. Brenner. 2004. Critical role of T cell-dependent serum antibody, but not the gut-associated lymphoid tissue, for surviving acute mucosal infection with *Citrobacter rodentium*, an attaching and effacing pathogen.J. Immunol. 172: 433-441.

De Grado M., C. M. Rosenberger, A. Gauthier, B. A. Vallance, and B. B. Finlay. 2001. Enteropathogenic *Escherichia coli* infection induces expression of early growth response factor by activating mitogen-activated protein kinase cascades in epithelial cells. Infect. Immun. 69: 6217-6224.

Ediger R. D., R. M. Kovatch, and M. M. Rabstein. 1974. Colitis in mice with a high incidence of rectal prolapse. Lab. Anim. Sci. 24: 488-494

Goncalves, N. S., M. Ghaem-Maghami, G. Monteleone, G. Frankel, G. Dougan, D. J. Lewis, C. P. Simmons, and T. T. MacDonald. 2001. Critical role for tumor necrosis factor alpha in controlling the number of lumenal pathogenic bacteria and immunopathology in infectious colitis. Immun. 69: 6651-6659.

Higgins, L. M., G. Frankel, I. Connerton, N. S. Goncalves, G. Dougan, and T. T. MacDonald. 1999. Role of bacterial intimin in colonic hyperplasia and inflammation. Science. 285: 588-591.

Higgins, L. M., G. Frankel, G. Douce, G. Dougan, and T. T. MacDonald. 1999. *Citrobacter rodentium* infection in mice elicits a mucosal Th 1 cytokine response and lesions similar to those in murine inflammatory bowel disease. Infect. Immun. 67: 3031-3039.

Itoh K., T. Matsui, K. Tsuji, T. Mitsuoka, and K. Ueda. 1988. Genetic control in the susceptibility of germfree inbred mice to infection by *Escherichia coli* O115a,c:K(B). Infect. Immun. 56: 930-935.

Klapproth J. M. A., I. C. A. Scaletsky, B. P. McNamara, L. C. Lai, C. Malstrom, S. P. James, and M. S. Donnenberg. 2000. A large toxin from pathogenic Escherichia coli strains that inhibits lymphocyte activation. Infect. Immun. 68: 2148-2155.

Luperchio S. A., J. V. Newman, C. A. Dangler, M. D. Schrenzel, D. J. Brenner, A. G. Steigerwalt, and D. B. Schauer. 2000. *Citrobacter rodentium*, the causative agent of transmissible murine colonic hyperplasia, exhibits clonality: synonymy of *C. rodentium* and mouse pathogenic *Escherichia coli*. J. Clin. Microbiol. 38: 4343-4350.

Luperchio S. A., and D. B. Schauer. 2001. Molecular pathogenesis of Citrobacter rodentium and transmissible murine colonic hyperplasia. Microb. Infect. 3: 333-340.

MacDonald, T. T., G. Frankel, G. Dougan, N. S. Goncalves, and C. Simmons. 2003. Host defences to *Citrobacter rodentium*. Int. J. Med. Microbiol. 293: 87-93.

Maggio-Price L., K. L. Nicholson, K. M. Kline, T. Birkebak, I. Suzuki, D. L. Wilson, D. Schauer, and P. J. Fink. 1998. Diminished reproduction, failure to thrive, and altered immunologic function in a colony of T-cell receptor transgenic mice: possible role of *Citrobacter rodentium*. Lab. Anim. Sci. 48: 145-155.

Malstrom C., and S. James. 1998. Inhibition of murine splenic and mucosal lymphocyte function by enteric bacterial products. Infect. Immun. 66: 3120-3127.

National Research Council. 1991. Infectious diseases of mice and rats: a report of the Institute of Laboratory Animal Resources Committee on Infectious Diseases of Mice and Rats. National Academy Press, Washington D.C.

Ocholi R. A., J. C. Chima, E. M. I. Uche, and I. L. Oyetunde. 1988. An epizootic infection of *Citrobacter freundii* in a guinea pig colony. Lab. Anim. 22: 335-336.

Pulliger, B. D. and S. Iversen. 1960. Mammary tumor incidence in relation to age and number of litters in C3Hf and RIIIf mice. Br. J. Cancer 14:267-278.

Schauer, D. B., B. A. Zabel, I. F. Pedraza, C. M. O'Hara, A. G. Steigerwald and D. J. Brenner. 1995. Genetic and biochemical characterization of *Citrobacter rodentium* sp. nov. J. Clin. Microbiol. 33:2064-2068.

Sellin J. H., S. Umar, J. Xiao, and A. P. Morris. 2001. Increased beta-catenin expression and nuclear translocation accompany cellular hyperproliferation in vivo. Cancer Res. 61: 2899-2906.

Silverman J., J. M. Chavannes, J. Rigotty, and M. Ornaf. 1979. A natural outbreak of transmissible murine colonic hyperplasia in A/J mice. Lab. Anim. Sci. 29: 209-213.

Simmons, C. P., N. S. Goncalves, M. Ghaem-Maghami, M. Bajaj-Elliott, S. Clare, B. Neves, G. Frnakel, G. Dougan, and T. T. MacDonald. 2002. Impaired resistance and enhanced pathology during infection with a noninvasive, attaching-effacing enteric bacterial pathogen, *Citrobacter rodentium*, in mice lacking IL-12 or IFN-gamma. J. Immunol. 168:1804-1812.

Vallance, B. A., W. Deng, K. Jacobson, and B. B. Finlay. 2003. Host susceptibility to attaching and effacing bacterial pathogen *Citrobacter rodentium*. Infect. Immun. 71: 3443-3453.

Vallance, B. A., W. Deng, L. A. Knodler, and B. B. Finlay. 2002. Mice lacking T and B lymphocytes develop transient colitis and crypt hyperplasia yet suffer impaired bacterial clearance during *Citrobacter rodentium* infection. Infect. Immun. 70: 2070-2081.

Authors: P. Kirsch / H. Meyer