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from the Working Group on Hygiene

Implication of infectious agents on results of animal experiments

Citrobacter rodentium

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Contents

Citrobacter rodentium

Background

- First isolated by Barthold et al. from an outbreak of mouse diarrhea in 1972[1]
- Formerly classified as *Citrobacter freundii* biotype 4280[2] and *Citrobacter genomospecies* 9[3]
- Later described by Schauer and Falkow in 1993[4] and renamed *Citrobacter* (*C.*) *rodentium* sp. nov.[5]

Prevalence

- Recent screening of 82.337 samples revealed no prevalence of *C. rodentium* in laboratory mice in North America and Europe.[6]
- Prevalence in wild mice is unknown.[7]

Host species

- *C. rodentium* is a natural murine intestinal pathogen.[2,8,9]

Properties

- *C. rodentium* is transmitted via the faecal-oral route and causes colitis.[10]
- *C. rodentium* is not highly contagious.[7]
- Bacteria that are shed at the peak of infection are hyperinfectious and are efficiently spread via coprophagy to uninfected littermates.[10]
- *C. rodentium* possesses a genetic pathogenicity island (LEE) and virulence factors that cause attaching and effacing (AE) lesions that are responsible for the pathogenesis of transmissible murine colonic hyperplasia (TMCH).[4,7,10-13]

Susceptibility

- *C. rodentium* appears to be highly species-specific: it is frequently associated with disease, when present in a mouse population.[7]
- Suckling mice, adult mice of some inbred strains[8,14,15], Han:NMRI mice[16] and transgenic lines[17] are more susceptible and demonstrate clinical signs.
- Germfree CF1 and C3H mice are highly susceptible, germfree C57BL/6 and NC mice are susceptible, and germfree BALB/C are resistant to infection.[1,4]
- Immunodeficient mice (mice lacking IL-12, IFN-γ, TNF receptor) and mice with deficiencies in cell-mediated immunity are more susceptible to infection than immunocompetent mice and infection is often fatal in immunodeficient mice.[18-23]
- Intestinal mucin (Muc2) deficient mice are highly susceptible to infection because of the disruption of the intestinal mucus layer.[24]
- Composition of the intestinal microbiota is an important factor that influences susceptibility to *C. rodentium* infection and the subsequent immune response.[10]
- Several nutrients like vitamin D, polyunsaturated fatty acids as well as deficiencies in selenium and vitamin E increase susceptibility to *C. rodentium* colonization.[10]
- One report about an epidemic outbreak in a gerbil colony[25]
- Rats and hamsters are not susceptible to infection.[8]

Organotropism

- *C. rodentium* is the etiologic agent of transmissible murine colonic hyperplasia (TMCH) in mice: descending colon is most commonly affected, but the entire colon and caecum may be involved.[2,26]
- *C. rodentium* primary colonizes the caecum, specifically within the caecal patch; following colonization of the caecum, *C. rodentium* establishes a colonic infection.[27]
- Clearance of *C. rodentium* in the faeces occurs 2-3 weeks post infection.[10]

Clinical disease

- Transient infection with no carrier state in immunocompetent mice lasting about 4 weeks[7,13]
- *C. rodentium* is often absent when clinical signs are apparent.[7]
- Clinical signs are nonspecific and include ruffled coat, weight loss, depression, stunting, perianal faecal staining, pasty dark faeces that smear the cage wall.[7,28]
- Rectal prolapse is frequently associated with TMCH.[7]
- Variable incidence of rectal prolapse in mice of all ages is indicative of infection.[28]
- Disease severity ranges from self-limiting colitis and immunity (that prevents an infection) to severe inflammation and potentially lethal dehydration.[10]
- Mice that recover are refractory to reinfection.[13]
- Wild-type mice clear the infection: T and/or B lymphocytes are required to clear the infection.[20]
- Survival and clearance of infection does not depend on secretory IgA or IgM antibodies, but passive immunization with serum IgG protects CD4-deficient mice from fatal infection.[23]
- Streptomycin in the drinking water may influence the severity of the disease.[29]
- *C. rodentium* has the ability to persist in mice following kanamycin treatment but not following vancomycin or metronidazole treatment.[30]
- Gut microbiota has a significant impact on *C. rodentium* disease course.[30]
- Age, host genetic background, diet, and indigenous microbiota influence disease expression.[9]
- Commercial diets effect the baseline colon morphology and presumably the epithelial cell turnover rate – the dietary constituents responsible for this effect are unknown.[8]

Pathology

- Mice develop colitis following infection.[31]
- Hallmark pathologic lesion: colonic hyperplasia with limited inflammation and epithelial cell hyperproliferation in the descending colon.[26]
- Characterized by crypt elongation, increased mitotic activity, mucosal thickening, variable mucosal inflammation, crypt abscesses, occasional erosions and ulcers, healing, and goblet cell hyperplasia[26,28]
- Grossly thickened, opaque and rigid distal colon, devoid of formed faeces[9,26]
- With increasing severity of disease, the entire colon, and less frequently, the caecum and ileum may be involved.[26]
- Caecum is often empty and contracted.[26]

- Hyperplasia is detectable as early as 4 days after infection, increases in severity through weeks 2 and 3, and resolves 5-8 weeks after infection.[26]
- Peak hyperplastic response occurs within 2-3 weeks with most prominent clinical signs.7
- Hyperplasia regresses with excessive goblet cell differentiation and development of cryptal cysts filled with mucin, mucosa returns to normal by 2 months.[8]
- Mucosal hyperplasia is more severe in outbred NIH Swiss mice as compared with C3H/HeJ, C57BL/6J and DBA/2J mice.[8]
- C3H/HeJ mice are more susceptible to disease than DBA/2J or C57BL/6J mice and develop a moderate degree of mucosal hyperplasia.[8]
- 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.[26]
- Necrosis of the colonic mucosa and severe colitis most notably in suckling mice[29]
- Young mice and certain mouse strains (C3H substrains, AKR, FVB) tend to develop more severe disease with varying mortality due to secondary inflammatory and ulcerative lesions in the hyperplastic mucosa.[7]
- C57BL/6 mice depleted of CD4+ T cells are highly susceptible to infection and develop severe colitis.[21]
- Mice lacking CD4+ T cells or B cells show hypersusceptibility to mucosal inflammation, colonic pathology, and systemic dissemination of *C. rodentium*.[20,32]
- 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[21]
- Infection of TLR2-deficient mice leads to severe colonic pathology, rapid weight loss and accelerated mortality.[33]
- Caspase 1-deficient mice and NLRP3-deficient mice have increased bacterial loads, aberrant inflammatory and chemokine responses, severe immunopathology and rapid weight loss.[10]
- Mucosal hyperplasia is dependent on the host immune response.[34]
- Hyperplastic responses do not occur in interferon (IFN)-γ receptor-deficient mice.[34]
- Depletion of IFN-γ prevents crypt hyperplasia.[35]
- CD4-/- or TCR-β-/- mice develop polymicrobial sepsis and end-organ damage (abscesses) and succumb during acute infection.[36]
- 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.[20]
- Mice devoid of B and T cells, CD4 T cells, or B cells fail to clear infection and succumb from sepsis, with bacteriaemia arising from *C. rodentium* and other gut bacteria.[7]
- Bacteriaemia and extra-intestinal infection are not hallmarks of infection, though recovery of bacteria from blood, liver and spleen has been reported.[9]
- 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.[36]

- Inflammatory and crypt hyperplastic responses in RAG1$^{-/-}$ mice are transient and infection is often fatal.[20]
- RAG1$^{-/-}$ mice respond to infection primarily with a granulocytic infiltration of the colonic mucosa.[20]

Morbidity and mortality

- Low morbidity and mortality in most adult mice, while mortality is observed in weaning-age mice.[8,26]
- Mortality is significantly higher in C3H/HeJ than in DBA/2J, Swiss, or C57BL/6 mice.[8]
- Increased level of mortality accompanied by a high incidence of rectal prolapse in outbred Swiss-Webster mouse[37]
- High mortality in T-cell receptor αβ transgenic mice[17]
- Mortality occurs from bacteriaemia, with multifocal hepatitis and splenitis in mice that are incapable of mounting effective immunity.[7]
- 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 did exhibit 45% mortality while no mortality was observed in Swiss-Webster mice.[9]

Zoonotic potential

- No data

Interference with research

Oncology

- *C. rodentium*-induced hyperplasia can alter chemical carcinogenesis in the large bowel.[8,38]
- Hyperplastic state of the colon serves as a promoter for colon tumorigenesis.[8]
- Transient hyperplastic state increases susceptibility to the carcinogenic effect of 1,2-dimethylhydrazine (DMH) in NIH Swiss mice: epithelial cell hyperproliferation promotes the development of colonic adenomas after administration of DMH.[39]
- DMH administration concomitant with hyperplasia reduces the latency period for early neoplastic lesions, however hyperplasia has no effect on already established tumors.[39]
- Hyperplastic lesions may be confused with neoplasia because associated cytokinetic alterations share several common features with those observed in neoplasia.[40,41]
- Apc$^{Min/+}$ mice infected with *C. rodentium* at 1 month of age have a 4-fold increase in the number of colonic adenomas in the distal colon at 6 months of age.[42]

Teratology

- No data

Infectiology

- *No data*

Immunology

- Colonic hyperproliferation is associated with cytokinetic alterations.[40]
- Expression of many proinflammatory cytokines is highly induced in response to *C. rodentium* infection.[43]
- *C. rodentium* infection causes a significant increase in the number of natural T_{reg} cells in the colon during active disease.[43]
- Infection generates a predominately lymphocytic infiltrate, characterized by CD4+ T cells situated near the proliferative epithelial crypts.[44]
- Increase of IL-10 secretion and inhibition of IL-2 and IL-4 secretion by mitogen-stimulated murine spleen cells[45]
- Variable effect on IFN-γ secretion, whereas the effect of enteropathogenic *Escherichia coli* lysates is inhibitory[45]
- Suppression of lymphocyte activation in vitro[46]
- Highly polarized Th 1 immune response in the gut, characterized by increased levels of IL-12, IFN-γ and TNF-α mRNA[44]
- *C. rodentium* triggers a potent Th17 cell response 1 week after oral challenge.[10]
- 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.[19]
- 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[19]
- MYD88 signalling controls *C. rodentium* infection: MYD88 deficiency compromises the ability of the host to restrict bacterial replication[10]
- Increased IL-17 and segmented filamentous bacteria (SFB) levels correlate with increased resistance to *C. rodentium*.[47]

Interactions with other infectious agents

- Probiotics are able to prevent *C. rodentium*-induced mortality in neonatal mice and are able to reduce *C. rodentium* colonization.[10]
- *Bacillus subtilis* slightly reduces colonic hyperplasia due to *C. rodentium* infection when administered to suckling Swiss NIH mice.[48]
- *Lactobacillus acidophilus* NCFM reduces colonic hyperplasia and inflammatory infiltrates due to *C. rodentium* infection in suckling BALB/cByJ mice.[49]
- Administration of a probiotic cocktail containing *Lactobacillus acidophilus* and *Lactobacillus rhamnosus* in C57BL/6J mice enhances bacterial killing, prevention of bacterial attachment, or enhancement of the Th1/T-regulatory response.[50]
- Coinfection with the intestinal helminth *Heligmosomoides polygyrus* can promote *C. rodentium*-associated disease and colitis in BALB/c mice and displays a marked morbidity and mortality.[51]
- Persistent *Helicobacter hepaticus* infection causes chronic *C. rodentium*-induced colitis in C57BL/6J mice, resulting in delayed recovery from weight loss and tissue damage.[43]
- *C. rodentium* infection modulates the outcome of fungal infection in the lung with *Cryptococcus neoformans* in C57BL/6 mice.[52]

- *C. rodentium* infection causes a pronounced dysbiosis that is characterized by an overgrowth of *C. rodentium* and a reduction in the abundance and overall diversity of the resident microbiota.[53]
- Transfer of the microbiota from resistant mice to mice that are susceptible to infection results in the transfer of host resistance to *C. rodentium* infection.[10]

Toxicology

- No data

Physiology

- *C. rodentium* infection causes alterations of phase I and phase II metabolic enzymes in liver and kidney, as well as increases in hepatic cytokine transcripts; distinct liver pathology correlated with serum elevation of liver transaminases, systemic and hepatic cytokine, and chemokine changes in C57BL/6 mice.[54]

Cell biology

- Increase in total cellular β-catenin accompanied by an increase in nuclear β-catenin concentrations; elevated levels preceded crypt elongation[55]
- Increase in cellular concentrations of cyclin D1 and c-Myc (proteins maintaining proliferation status)[55]
- Increased transcription of EGR-1 with subsequent activation of the MEK/extracellular signal-regulated kinases[56]
- Increase in production of keratinocyte growth factor, which induces cell proliferation[44,57]

Assisted reproductive technology

- No data

Special considerations

- The *C. rodentium* mouse model is the gold standard for studying virulence of the closely related human attaching and effacing enteropathogenic *Escheria coli* (EPEC) and enterohemorrhagic *Escheria coli* (EHEC).[10,23,58]
- *C. rodentium* infection is used to model several human intestinal disorders like Crohn's disease, ulcerative colitis, and colon tumorigenesis.[10]
- *C. rodentium* is a tool to investigate adaptive mucosal immune responses to bacterial infection.[10,23]
- *C. rodentium* infection is a reproducible, robust, and physiologically relevant model of inflammation.[54]

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