GV-SOLAS
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Expert Information

from the Working Group on Hygiene

Implication of infectious agents on results of animal experiments

Murine Norovirus

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Contents

Murine Norovirus (MNV)

Background

- Non-enveloped single-stranded RNA virus[1]
- Family *Caliciviridae*, genus Norovirus[1]
- Norovirus first detected in humans after an outbreak of acute gastroenteritis in a school in Norwalk (Ohio, USA) in 1968.[1]
- There is only one species within the genus, Norwalk virus, but more than 100 strains (ICTV).
- First description of a “Norwalk-like” virus in mice, murine norovirus (MNV)[1], in 2003[2]
- In 2006, three new murine norovirus strains were identified (MNV-2, MNV-3, MNV-4)[3]
- Meanwhile, many additional strains of MNV were isolated[1] and over 100 different MNV sequences are currently deposited in GenBank.[4]
- Besides multiple murine norovirus strains, also rat, bovine, ovine, canine, and porcine noroviruses were described.[1,4,5]
- MNV mouse models are important models for human norovirus research.[6]

Prevalence

- In a study conducted by one of the leading commercial rodent diagnostic laboratories 32% of the mouse serum samples submitted from institutions in North America and Europe over several years had antibodies against MNV, the most prevalent agent in this study.[7]
- In a study from 2009, the prevalence in European research facilities was similar with 31.8%.[8]

Host species

- Natural host of the virus is the mouse.
- MNV is detected in wild and laboratory mice.[9]
- Murine norovirus is endemic in many research colonies.[1]

Properties

- Excretion of virus in feces[10,11]
- MNV is sufficiently transferred to sentinel mice via soiled bedding[1,10,12]
- Several strains of the virus are able to persist in various tissues (small intestine, caecum, mesenteric lymphnodes, spleen) of immunocompetent (C57BL/6J, Hsd:ICR(CD-1), Jcl:ICR) and immunodeficient mice (CB17-PrkdcScid) mice, with virus shedding in feces or persistence in organs for at least 35-60 days.[1,3]
- Persistent infection of RAG2-deficient mice[2,12]
- In one research institute, ten distinct murine norovirus strains were isolated from tissues and feces of asymptomatic wild type and immunodeficient mice; isolates were distinguishable according to the specific location of the facility.[13]
- In the same study, the comparison of the amino acid sequences showed, that the proteinase was the most highly conserved protein, while the VP1 (major capsid

protein) showed the most variability; within VP1, the shell (S) region was conserved among all virus strains isolated, while the protruding region (P) was most variable.
- Different MNV strains are considered to belong to the same genotype and genogroup, with an extreme level of conservation of the ORF1-ORF2 (open reading frames) junction; the genogroup seems to be a single serotype.[14]
- Serologic cross-reactivity was detected between MNV-1 and MNV-2, MNV-3 and MNV-4, isolated from geographically separate mouse research colonies.[3]

Susceptibility

- Wild, pet and laboratory mice[4,9]
- Experimental infections with MNV-1 showed that the duration of infection and clinical manifestation is dependent on the mouse strain.[2,15,16]
- Neonatal Swiss Webster mice were resistant to MNV infection after experimental oral inoculation on day 1-3 post partum (p.p.), but susceptibility developed by day 5 p.p.[17]

Organotropism

- After experimental infection (oral inoculation), MNV was isolated from caecum, duodenum, liver, and spleen in Scid and ICR mice, but not from brain, heart, kidney, lung, salivary gland, thymus, ovary, or uterus.[18]
- Immune cells are most likely target cells of MNV.[4]
- MNV infects macrophages and dendritic cells in culture and *in vivo*.[4,19]
- MNV shows a tropism for macrophages and dendritic cells in immunodeficient mice.[20]
- MNV also infects B cells in culture, and studies in mice lacking functional B cells corroborate B cells to be a target also *in vivo*.[4]

Clinical disease

- Duration of infection and disease manifestation vary depending on mouse and virus strain.[1,4]
- In immunocompetent mouse strains infection is variable in length, but without clinical signs of disease.[1]
- In certain immunodeficient strains, infection can cause lethal systemic disease with encephalitis, vasculitis, meningitis, hepatitis, and pneumonia (e.g. interferon-deficient mice, $Stat^{tm1}$ mice) or it can persist ≥ 90 days without symptoms (e.g. $Rag1^{-/-}$, $Rag2^{-/-}$).[1]
- Clinical signs such as weight loss, ruffled fur and hunched posture were noted in naturally infected immunodeficient mice strains with C57BL/6 background ($Rag1^{-/-}$/IFNγ$R^{-/-}$ mice, $Rag1^{-/-}$/$Stat1^{-/-}$ mice, OT1 $Rag1^{-/-}$/IFNγ$R^{-/-}$ mice, and OT2 $Rag1^{-/-}$/IFNγ$R^{-/-}$ mice); no clinical symptoms or lesions were observed in $Rag2^{-/-}$ mice, which indicates that the innate immune system plays an essential role in MNV infection and is critical for resistance to MNV-1.[20]
- Clinical disease is controlled or prevented by STAT1-dependent interferon responses, including direct inhibition of viral replication and prevention of virus dissemination.[15]
- Experimentally infected mice deficient for STAT1 innate immune pathway genes develop encephalitis, meningitis, cerebral vasculitis, focal interstitial pneumonia, peritonitis, pleuritis, and hepatitis.[2]

- MNV-1 is the prototype for acute infection, whereas MNV-3, -4, CR-3 and CR-6 are considered to be persistent strains.[4]

Pathology

- Mild histopathological alterations in the small intestine (increase of inflammatory cells) and spleen (red pulp hypertrophy and white pulp activation) in 129S6 mice[1,15]
- Encephalitis, vasculitis, meningitis, hepatitis, and pneumonia are detected in e.g. interferon-deficient mice, Stat tm1 mice.[1]
- Natural infection with MNV causes inflammation of multiple tissues (hepatitis, focal interstitial pneumonia, peritonitis, pleuritis) in association with clinical signs as well as asymptomatic infection of mesenteric lymph nodes in immunodeficient mice strains (see above).[20]
- In a study, immunocompetent MNV-antibody positive female Swiss Webster sentinel mice did not exhibit clinical signs or gross lesions; histopathologically, only a few hepatic inflammatory foci were found, some of which were immunoreactive with antibodies to MNV.[11]
- A review in 2016[21] concluded that MNV in wild-type mice caused no to minimal histologic changes, whereas in immunodeficient mouse strains MNV-induced lesions were consistently present, including inflammatory lesions in the liver, as well as variable lesions in the lung, gastrointestinal tract, mesenteric lymph nodes, brain, and spleen.

Morbidity and mortality

- Depending on virus and mouse strain, see above under Clinical disease

Zoonotic potential

- No data

Interference with research

Oncology

- No data

Teratology

- No data

Infectiology / Interactions with other infectious agents

- Upregulation of antiviral genes[4]
- In a study from Compton et al. (2010) MNV infection enhanced a subsequent mouse parvovirus (MPV) infection in BALB/c mice, which seroconverted to MPV more rapidly and had substantially higher DNA levels in several tissues and feces, and extended duration of MPV shedding.[22]

- Exposure to MNV-1 resulted in a decreased CD8 T cell response to immunodominant MCMV (murine cytomegalovirus) epitopes in both BALB/c and C57BL/6 mice, whereas it did not affect MCMV titres in both mouse strains.[23]

Immunology

- MNV-1 replicates in macrophages and dendritic cells.[1]
- MNV infects hematopoietic cells and alters their cellular morphology.[19,20]
- Tropism for innate and adaptive immune cells may influence models of inflammatory diseases, especially intestinal inflammatory conditions.[4]
- Persistent MNV infection can have immunomodulatory effects on the mouse model used for Inflammatory Bowel Disease IBD (Atg16L1) and cause changes in the intestinal abnormalities.[4,24]
- MNV induces inflammation in the IL10-deficient mouse model of IBD[25]
- MNV has the potential to shape the host mucosal immunity under certain conditions.[4]
- Experimental infection of NOD mice (non-obese diabetic mouse model) with MNV-4 induces significant changes in mucosal immunity (cytokine secretion, antiviral immune signalling markers, concentration of mucosal antibodies) as well as systemic immunity (alteration of B cell subsets, macrophages, and T cells).[26]

Toxicology

- No data

Physiology

- MNV provides beneficial effects of commensal bacteria in germ-free mice and can restore intestinal health similar to bacterially colonized mice (villus width, lymphocyte number and function, antibody levels, basal suppression of innate lymphoid cell expansion) and may be considered a commensal in nature.[4]
- MNV infections may alter the intestinal microbiota under certain conditions4; in one study, experimental infection of two wild-type mouse strains, outbred Swiss Webster and inbred C57BL/6, with MNV strains causing acute (MNV-1) or persistent (MNV-4 and CR6) infection did not cause major disruptions in the murine intestinal microbiota (tissue-associated or fecal-associated bacterial communities)[27], whereas in another study comparing the effect of malnutrition and MNV1infection in C57BL/6 mice, an independent effect of MNV-1 on the composition of the microbiota was shown.[6]
- Experimental infection with MNV-4 protects NOD mice against the development of type 1 diabetes, associated with effects on T cells (expansion of T regs and reduction of proinflammatory T cells).[26]
- In the same study and mouse model, it was also found that MNV-4 significantly modified the gut commensal bacteria composition (increased α-diversity and Firmicutes/Bacteroidetes ratio).[26]
- MNV infection exacerbates atherosclerosis in Ldlr−/− mice fed a high-fat diet but does not influence obesity- and diabetes-related phenotypes; furthermore, the increased lesion size was associated with increased macrophages, suggesting that MNV may influence macrophage activation or accumulation in the lesion area.[28]

Cell biology

- No data

Assisted reproductive technology

- No data

Special considerations

- A case report from 2008 about eradication strategies describes that the test-and-removal method (selective testing followed by removal of positive animals) was ineffective in eliminating MNV, compared to depopulation and cleaning, that resulted in no evidence of MNV thereafter.[12]
- Murine norovirus infection is prevented, and decontamination successfully achieved by cross-fostering of neonatal mice, in combination with sanitary measures.[17,29]
- Autoclaving and dry-heat sterilization of cages and bedding prevents the transmission of MNV to naïve mice.[30]
- MNV-4 is detectable in soiled bedding sentinel mice (CD-1) in pooled fecal samples (1:10 or 1:20), and after storage of feces at room temperature for up to two weeks.[10]

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