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Customer protocol

Determination of bacterial load from tissues infected with *Citrobacter rodentium*

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Background

Citrobacter rodentium (*C. rodentium*) colonizes the mouse gastrointestinal system and is a rodent equivalent of human enteropathogenic *Escherichia coli* infection.
The organization and maintenance of mature colonic patches (CLPs) and isolated lymphoid follicles (ILFs), during infection with *C. rodentium*, has been analyzed.
It was shown that interleukin 22 (IL-22) acts downstream of the lymphotoxin pathway and regulates the organization and maintenance of CLPs and ILFs during infection with *C. rodentium*. IL-22 is sufficient to restore the organization of CLPs and ILFs and host defense against the infection with C. rodentium in mice lacking lymphotoxin signals.
The results suggest that IL-22 connects the lymphotoxin pathway with epithelial defense mechanisms.[1]
This protocol describes the procedure to homogenize mouse spleen and mouse liver tissue by the gentleMACS™ Dissociator that allows a final determination of *C. rodentium* bacterial burden.

Materials and methods

Materials

- gentleMACS Dissociator or gentleMACS Octo Dissociator
- gentleMACS C Tubes
- Incubator (37 °C)
- Phosphate-buffered saline (PBS) with 0.1% Triton® X-100
- MacConkey agar

Methods

1. Remove the spleen and liver from the mouse.
2. Weigh organs.
3. Transfer the whole organ into the gentleMACS C Tube containing 3 mL (for liver) or 2 mL (for spleen) of PBS with 0.1% Triton X-100.
   (Note: The viability of the bacteria can be further increased using PBS with 0.2% NP40 instead of 0.1% Triton X-100.)
4. Tightly close the C Tube and attach it upside down onto the sleeve of the gentleMACS Dissociator.
5. Run the gentleMACS Program **m_spleen_01** for both tissues.
6. Serially dilute the homogenates (1:5 for liver and 1:3 for spleen) using PBS with 0.1% Triton X-100.
7. Spot 5 µL of each suspension on pre-dried MacConkey agar plates in triplicate.
8. Incubate plates overnight at 37 °C.
9. Determine colony-forming unit (CFU) counts.

Results

Treatment of mice with lymphotoxin β receptor Fc (LTβR-Fc) resulted in the systemic dissemination of *C. rodentium* into the spleen and liver whereas the treatment with IL-22-Fc significantly diminished the spread of the bacteria into both organs.

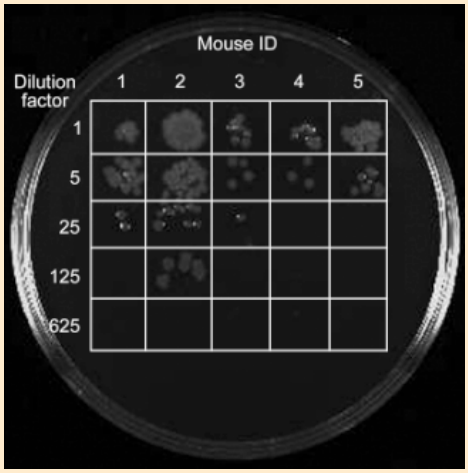

Figure 1: Mice organs were removed and treated as described in the method section. Different dilutions of the liver homogenates were plated on MacConkey agar.

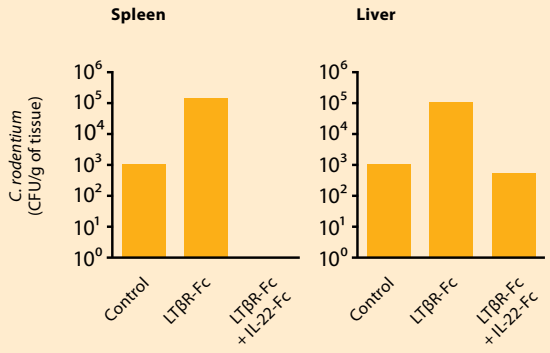

Figure 2: Quantification of bacterial burden in the spleen and liver of mice treated as described above.

Conclusion

C. rodentium bacterial load analysis can be accomplished with ease using the gentleMACS Dissociator.

Reference

1. Ota, N. *et al.* (2011) IL-22 bridges the lymphotoxin pathway with the maintenance of colonic lymphoid structures during infection with *Citrobacter rodentium*. Nat. Immunol. 12: 941–948.

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