EXAM TU 02: Infectiology
Virology

Session: 1st (2023-2024)

DATE: 2nd of May 2024
Duration: 1 hour
No authorized document, no calculator

Synthesis question (7 points)

Virus structure and modes of transmission.

1. What are the different structures that make up viruses? Describe their functions.
2. Explain how the virus structure affects the modes of transmission of viruses.

You **must** illustrate your answer with schemas.

Short open-ended question (3 points)

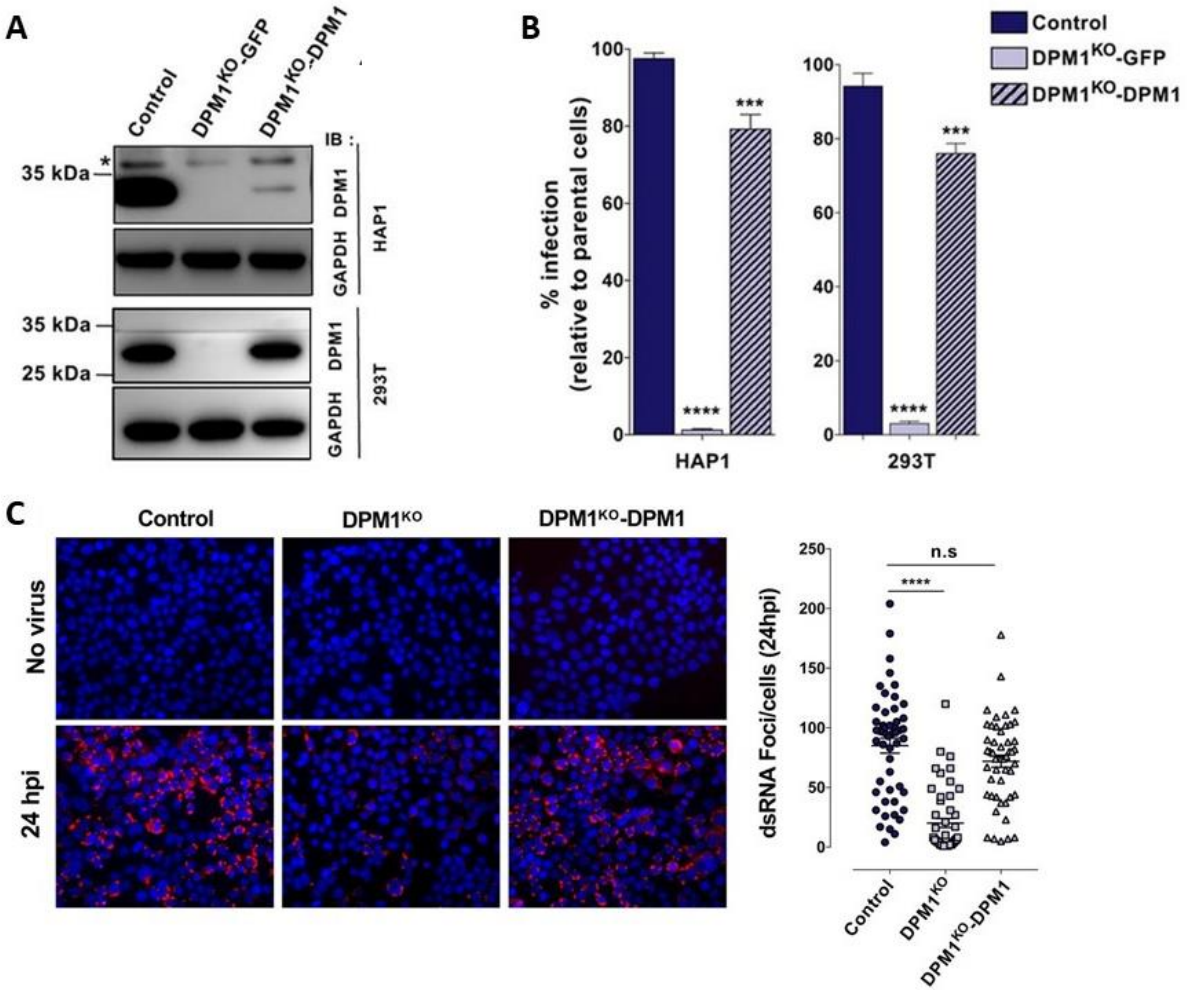
1. What are the stages of HIV infection?

2. Explain why a negative-stranded virus needs to carry its own polymerase in the viral particle.

3. Influenza virus subtype H5N1 is responsible for serious infections in birds. Explain what the letters H and N stand for. Which influenza virus subtypes are generally found in humans?

Figure analysis (10 points)

Using a CRISPR-Cas9 screen in haploid HAP1 cells, the cellular protein DPM1 was identified as a potential host dependency factor for dengue virus (DENV). In the **following figure**, more experiments were performed in HAP1 and 293T cells, in order to confirm and investigate the role of this protein in DENV infection.



(A) Immunoblot of DPM1 in control and DPM1^{KO} cells trans-complemented with the respective cDNA.
(B) 293T and HAP1 trans-complemented clones were challenged with DENV (MOI of 5 in 293T cells and MOI of 10 in HAP1 cells). Infection was quantified 48 hours post-infection (hpi) by flow cytometry using an antibody against one of the envelop protein of the virus. Parental cells are naive HAP1 or 293T cells without CRISPR-Cas9.
(C) Control, DPM1^{KO}, and DPM1^{KO} 293T cells complemented with DPM1 were inoculated with DENV (MOI of 100). Left, representative images of the cells stained with anti-dsRNA antibody (red) and DAPI (blue) at 24 hpi. Right, quantification of the number of foci per cell using Icy software.

Data are means ± SD from a representative experiment with n = 50 independent cells. Significance was calculated using a one-way ANOVA with Dunnett's multiple-comparison test. ****, P < 0.0001.

(FYI: DPM1 is a subunit of the dolichol-phosphate mannose (DPM) synthase complex (DPMS), which transfers a mannose residue from the GDP-mannose donor to the dolichol-phosphate carrier in pathways leading to N-glycosylation, glycosylphosphatidylinositol (GPI) anchor biosynthesis, and C- or O-mannosylation of proteins in the ER lumen)

Questions:

1) Describe the experiment performed in **panel A** (type of experiment, purpose, conditions, readout, controls, etc.) and conclude on the results.
2) What is a trans-complementation experiment? Why is it important? In your opinion, why did they use two different cell lines?
3) Describe the experiment performed in **panel B**. Specifically, explain what is measured, how it is measured and how the results are expressed on the graph.
4) Conclude on the results of **panel B.**
5) Describe the experiment performed in **panel C.** What is the purpose of using an anti-dsRNA (double-stranded RNA) antibody? Why do they use DAPI?
6) Conclude on the results of **panel C**.
7) Can you give a general conclusion about the whole figure? Following those first results, does DPM1 seem to have a proviral or an antiviral role during the DENV cycle?

Bonus question: Propose one follow-up experiment to go further on understanding the role of this protein in DENV infection.