Reporter assay-based cell line for rapid and early determination of vaccine-induced protection against foot-and-mouth disease virus

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Key words
FMDV, protective antibody measurement, FcR-bearing cell line reporter assay, diagnosis/surveillance

Aim of the study
Foot-and-mouth disease virus (FMDV) is a highly transmissible picornavirus affecting cloven-hoofed animals, causing a great threat to any country with strict trade and animal movement restrictions in case of outbreaks. In endemic regions, vaccination is used routinely whereas in FMDV-free regions vaccination is only permitted as an emergency measure. The potency tests to estimate vaccine efficiency are carried out by performing cattle challenge tests, which are ethically problematic, costly and do not have a strong statistical power. Thus, reliable in vitro correlates of vaccine-induced protection are urgently required. The present project links with the EU-funded project FMD-DISCONVAC to develop in vitro assays to avoid challenge infection of cattle for the purpose of FMD vaccine testing and selection. To this end a cell-based assay system to permit the measurement of opsonizing antibodies was developed and evaluated.

Material and methods
Sera from cattle vaccinated with commercial FMDV vaccines and monoclonal antibodies against O, A and SAT-2 isolates serotype were generated ourselves or obtained from DISCONVAC partners. Bovine FcγRI (CD64), RII (CD32) and RIII (CD16) were cloned in a lentivirus expression system and murine RAW 264.7 macrophages cell lines expressing the bovine Fc receptors were generated. These cell lines were employed to measure antibody mediated enhanced infection by FMDV which was quantified in terms of virus-induced cytopathogenic effects using flow cytometry or incubation of cells with WST-1, followed by a spectrophotometric assay of the colored product as a measurement of cellular metabolic activity.

Results and significance
We have established a test system to measure opsonizing antibodies using a bovine CD32 expressing RAW 264.7 cell line which is resistant to FMDV infection unless the virus is opsonized with serum. Opsonizing activity was observed against homologous serotype strains with a broader level than neutralizing activity. Cross-reactivity was also seen against some heterologous serotype strains. In general opsonization was still measurable with at least 10 times lower serum dilution as compared to neutralizing titres. Using monoclonal antibodies we determined the existence of epitopes mediating opsonization under conditions where neutralization does not take place. Generally, also with monoclonal antibodies opsonizing activity was much broader in terms of reactivity with many isolates and even serotypes compared to neutralizing activity. Interestingly a better correlation to the vaccine dose was observed with opsonizing antibody levels when compared to neutralizing antibody titers. Future studies with more serum samples are required to determine the value of opsonizing antibody quantification as a correlate of vaccine-induced protection against FMDV.

Publications, posters and presentations
One manuscript in preparation.
The work will be presented at the Open Session of the EU-FMD meeting in Jerez, Spain, October 29-31, 2012.

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