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Research paper

Discrimination within epitope specific antibody populations against Classical swine fever virus is a new means of differentiating infection from vaccination

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ABSTRACT

Serological differentiation between infection and vaccination depends on the detection of pathogen specific antibodies for an epitope that is modified or lacking in a vaccine. Here we describe a new assay principle that is based on differences in the binding properties of epitope specific antibodies. C-DIVA is a potent Classical swine fever vaccine candidate that differs from the parental C-strain life attenuated vaccine in the highly immunogenic TAVSPTTLR epitope by the deletion of two and the mutation of one amino acid (TAGSΔΔTLR). We show that C-DIVA vaccination elicits antibodies with high affinity for both the TAGSΔΔTLR and TAVSPTTLR epitope, whereas infection elicits only TAVSPTTLR specific antibodies. Differentiation is achieved with a double competition assay with negative selection for antibodies with affinity for the TAGSΔΔTLR epitope followed by positive selection for antibodies with affinity for the TAVSPTTLR epitope. Our findings add a new strategy for the development of marker vaccines and their accompanying discrimination assays and offer an alternative to the devastating stamping out policy for Classical swine fever.

1. Introduction

The inability to distinguish between infected and vaccinated individuals is an intrinsic limitation to the use of vaccines. This can be overcome by marker vaccines in combination with matching diagnostics that discriminate infection and vaccination by marker dependent differences in the immune response. However the immune response against added positive markers is not indicative of the quality of the vaccination. Alternatively the creation of a negative marker of sufficient sensitivity requires the elimination or modification of a highly immunogenic epitope with the risk of reduced potency and viability of such vaccines (Dong and Chen, 2007; Eblé et al., 2013; Reimann et al., 2010).

Here we describe a new marker principle applied to Classical swine fever (CSF). CSF is a highly contagious disease causing enormous financial losses and the mandatory pre-emptive slaughter of millions of pigs (Meuwissen et al., 1999; Paton and Greiser-Wilke, 2003; Stegeman et al., 2000). Live attenuated vaccines against CSF provide full protection within a few days after vaccination but have been no alternative to the stamping out policy because they do not fulfill the DIVA (Discrimination between Infected and Vaccinated Animals) criteria (Qiu et al., 2006; Schroeder et al., 2012). Only one CSF epitope, the linear TAVSPTTLR epitope meets the criteria of specificity and sensitivity as a target for serologic assays (Eblé et al., 2013; Schroeder et al., 2012). The TAVSPTTLR epitope is

Abbreviations: C-DIVA, China strain derived marker vaccine; CSF, Classical swine fever; DIVA, Differentiation between Infected and Vaccinated Animals; TAGSΔΔTLR, modified TAVSPTTLR epitope in C-DIVA; TAVSPTTLR, epitope of CSF E2.

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unique because it is highly specific for CSF virus, highly conserved amongst all known CSF strains and highly immunogenic (Kortekaas et al., 2011; Lin et al., 2000; Liu et al., 2006). All vaccines with a modified or replaced TAVSPTTLR moiety still elicit antibodies interfering in the detection of TAVSPTTLR specific antibodies (Kortekaas et al., 2011; Reimann et al., 2010; Schroeder et al., 2012) which demonstrates the difficulty of discrimination on the basis of specificity for TAVSPTTLR. This dilemma demands either a further search for a new efficacious vaccine that does not elicit these interfering antibodies or a search for a new means of discrimination between TAVSPTTLR specific antibody populations elicited by infection and those elicited by an existing vaccine. Focusing on the latter we re-examined antibody populations elicited by an established live attenuated vaccine known as C-strain containing the wild type TAVSPTTLR epitope, and a derivative vaccine differing from the parental strain in the TAVSPTTLR epitope called C-DIVA. C-strain mutants with progressively larger deletions of the TAVSPTTLR epitope resulted in highly reduced viability. C-DIVA is the result of passaging C-strain mutants in persistently infected cells allowing the increase of their fitness by forced evolution (Kortekaas et al., 2011).

2. Materials and methods

2.1. Sera

Previously described sera (Kortekaas et al., 2011) from vaccination/challenge experiments and sera from the same experiment, collected 70 days post vaccination, were provided by J. Kortekaas, (Virology Division, Central Veterinary Institute, part of Wageningen University Research Center, Lelystad, The Netherlands). Negative field sera from the Netherlands and sera from animals experimentally infected with several CSFV, BVDV, and BDV strains were provided by W.L. Loeffen, (Virology Division, Central Veterinary Institute, part of Wageningen University Research Center, Lelystad, The Netherlands). Sera from experimental infection of pigs included the CSF strains Alfort, Behring, Bergen, Brescia, Henken, Isol. Rusland, Melis, Paderborn, Spruit, and v. Zoelen.

2.2. Recombinant E2 molecules

The E2 genes of vFlc34 (C-strain) and vFlc-ΔPTa1 (C-DIVA), codon optimized for expression from baculovirus vectors, were chemically synthesized and cloned behind the polyhedrin promoter in a pFastBac plasmid. The expression cassette was transferred by site-specific transposition in DH10Bac *E. coli* cells to a recombinant bacmid DNA in which chitinase and v-cathepsin genes had been deleted. Bacmid DNA was used to transfect Sf9 insect cells. Recombinant baculovirus obtained from the transfection supernatant was used to infect fresh Sf9 insect cells. Subsequent virus stocks were used to infect Sf21 insect cells cultured in 2 L bioreactors. Both recombinant proteins were primarily expressed in the supernatant of these bioreactor cultures. Both recombinant E2 proteins were affinity purified using purified monoclonal antibody C2 (Kortekaas et al., 2010) coupled to Sepharose. E2 in supernatants was quantified by an ELISA measuring the inhibition of C2 binding to purified E2 on the solid phase by E2 in the liquid phase.

2.3. Assays

IDEXX CSFV Ab Test Kit: Tests were performed according to the instructions of the manufacturer (IDEXX Livestock and Poultry Diagnostics, Liebefeld-Bern, Switzerland).

Binding property assessment assay: The assay measures the affinity of polyclonal serum antibodies for either the TAVSPTTLR epitope of the recombinant C-strain E2 or the TAGSΔΔTLR epitope of the recombinant C-DIVA E2 (Fig. 1). It differs from the IDEXX CSF Ab Test kit only in that the sera are incubated in the presence of recombinant E2 in the liquid phase. This incubation step contains mixtures of 40 µl sample diluent, 20 µl serum, and 40 µl purified recombinant E2 molecules. Each serum was separately incubated in the presence of differing concentrations (600 µg/ml, 10 µg/ml, 3.33 µg/ml, 1.11 µg/ml, 0.37 µg/ml, 0.12 µg/ml, 0.041 µg/ml) of either C-strain E2 and C-DIVA E2 diluted in PBS/BT (PBS containing BSA (0.1% w/v) and Tween 20 (0.05% v/v)) or PBS/BT as a control. In each plate a serial dilution of conjugate (100%, 50%, 25%, and 12.5% of the undiluted conjugate) was added in wells that had been incubated with PBS/BT during the first incubation step. The degree of inhibition due to the competition between

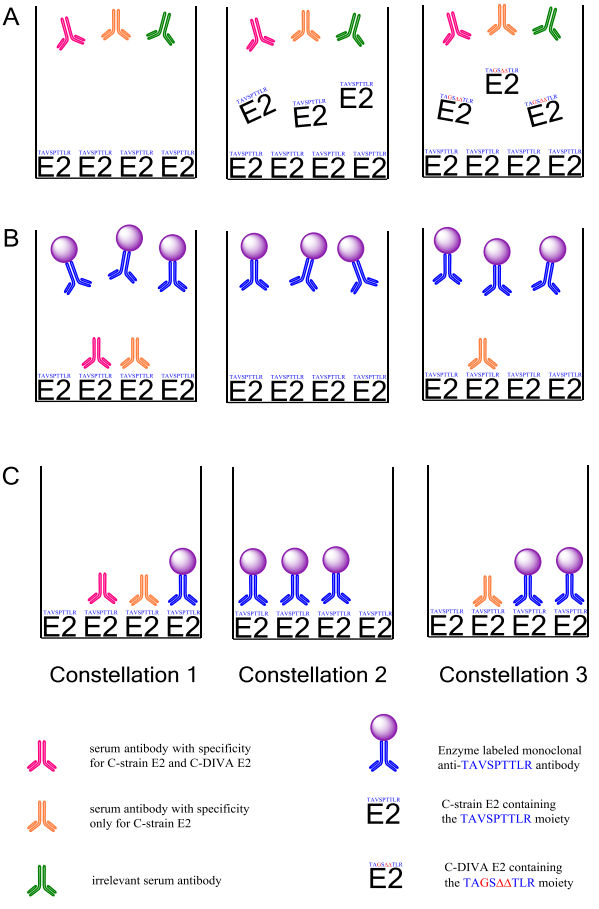

Fig. 1. Assessment of TAVSPTTLR specific antibody reactivities. Schematic diagram of TAVSPTTLR specific antigen antibody interactions. Constellation 1 depicts a standard CSF assay based competition between serum and monoclonal antibodies. Constellations 2 and 3 depict the reversal of the inhibition shown in Constellation 1 due to competition between E2 in the solid phase and in the liquid phase for binding to serum antibodies.

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