P.014 ANTIGENIC DIFFERENCES AMONG PCV2 STRAINS OF DIFFERENT GENETIC CLUSTERS AS DEMONSTRATED BY THE USE OF MONOCLONAL ANTIBODIES GENERATED AGAINST PCV2A OR PCV2B

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Introduction

Porcine circovirus type 2 (PCV2) is the causal agent of postweaning multisystemic wasting syndrome (PMWS) in weaned pigs (1). A recently proposed classification system divides the PCV2 strains into two major genotypes (PCV2a and PCV2b) and 8 genetic clusters: 1A to 1C (PCV2b) and 2A to 2E (PCV2a) based on their genomic sequences (2, 3). Lefebvre et al. (4) confirmed antigenic differences between different PCV2 strains by using mouse monoclonal antibodies (mAbs) raised against PCV2a. In that study, only a limited number of PCV2 strains with only 3 out of 8 genetic clusters were enclosed. To date, no studies have been performed to identify antigenic differences between different PCV2 strains including all genetic clusters of both genotypes PCV2a and PCV2b by using mAbs raised against both genotypes PCV2a and PCV2b. Thus, the present study aimed to identify antigenic differences between PCV2 strains of all genetic clusters by using mAbs raised against both PCV2a and PCV2b.

Materials and Methods

Fourteen different PK-15 cell-adapted PCV2 strains of all eight PCV2 clusters and with different clinical and geographical origin were used in this study. 1A/1B strains: 48285, 1147, II9F, 1206; 1C: NL_Control_4, II11A; 2A: Aust 10; 2B: Pingtung-1 and Pintung-4; 2C: PCV2 390 (kindly provided by Dr. Monica Balasch); 2D: Tomasz (isolated in our lab) and 2E: Stoon-1010, 1121 and 1103 were enclosed.

Sixteen mouse monoclonal antibodies (mAbs) (9C3, 13H4, 16G12, 21C12, 31D5, 38C1, 43E10, 48B5, 55B1, 59C6, 63H3, 70A7, 94H8, 103H7, 108E8 and 114C8) were generated against genotype PCV2a strain Stoon-1010 (4) and 6 mouse mAbs (6E9, 12E12, 14G2, 19C1, 19G10 and 22C1) were generated against genotype PCV2b strain 1147. All 14 PCV2 strains mentioned above were used to make 96-well IPMA plates as described by Saha et al. (5). PCV-negative PK-15 cells and the persistently PCV1 infected PK-15 cell line were used for control IPMA plates. The staining procedure was similar to the IPMA technique described by Lefebvre et al. (4) with ten-fold serial dilutions of hybridoma supernatants (mAbs) in PBS used as primary Abs. IPMA antibody titres of a hybridoma supernatant were expressed as the reciprocal of the last dilution that resulted in a positive reaction. These assays were performed independently for 3 times for each strain.

Results

Four out of 22 mAbs reacted with all PCV2 strains with the titres ranging from 10 to 10,000 (12E12, 21C12, 38C1 and 114C8). One mAb, 19G10 stained all PCV2 strains except the strain 390 which belongs to cluster 2C. MAbs 6E9, 9C3, 16G12, 43E10, 55B1, 63H3, 70A7, 94H8 and 103H7 did not react with the PCV2 strains of clusters 1C, 2B, 2C and 2D or they had IPMA Ab titres to these strains at least 100 times lower than for the PCV2 strains of clusters 1A/1B, 2A and 2E. Only two mAbs (14G2 and 19C1) were found to be specific for 1A/1B strains, although mAb 19C1 showed very low IPMA antibody titres (1 to 10) to 1A/1B strains. MAbs 31D5, 59C6 and 108E8 had titres ranging from 1,000 to 10,000 for 2A and 2E strains and did not react to the other strains or they had IPMA antibody titres at least 100 times lower than for the 2A and 2E strains. MAb 22C1 did not react or showed very weak reaction with PCV2a strains (2A to 2E) but clearly reacted with all PCV2b strains (1A to 1C, except strain NL_Control_4) and IPMA antibody titres were at least 100 times higher to PCV2b strains than for the PCV2a strains. MAb 13H4 stained only Stoon-1010 (PCV2a 2E) but did not stain or had very low titres to all other PCV2a or PCV2b strains. MAb 48B5 reacted with all PCV2a strains (except 390, type 2C strain) but only one PCV2b strain, 1206 (type 1A/1B). None of the 22 mAbs reacted with PCV1 infected cells or PCV-negative PK-15 cells.

Discussion

This study demonstrates that the different genetic clusters can be discriminated by using a large panel of mAbs. The findings might have consequences for the development of diagnostic assay. Four universal mAbs were reactive to all PCV2 strains. Some mAbs were cluster-specific. The results of this study may be indicative for possible binding sites of different mAbs in the capsid protein.

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