

Development of Monoclonal Antibodies for Universal Use in the Diagnosis of PRRS

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Summary and Implication

A panel of seven different groups of monoclonal antibodies (Mabs) specific for the 15 kD nucleocapsid protein of porcine reproductive and respiratory syndrome virus (PRRSV) was generated from mice that were immunized with antigens derived from PRRSV isolate ATCC VR-2402 (ISU-P). These groups, designated A through G, were determined by their reactivity in the IFA to 67 different field isolates representing 13 states, Canada, the European Lelystad virus and the current North American vaccine strain, ATCC VR-2332. The field isolates were collected during 1989 through 1995. Group A Mab's reacted with all field isolates and the Lelystad virus and as such should be considered for use as universal diagnostic Mab's for PRRSV. The field isolates and the Lelystad virus were able to be categorized into seven distinct groups (I-VII) based on their reactivity to the panel of Mab's. Group I represented 56 isolates. The six remaining groups were represented by one to four field isolates each. These observations demonstrate that there is a wider degree of diversity among North American PRRSV isolates than was previously believed.

Introduction

Porcine reproductive and respiratory syndrome (PRRS) is an economically important viral disease of swine caused by a small, enveloped RNA virus of the family *Arteriviridae* (4). Clinical manifestations of PRRSV infection are characterized by reproductive failure in sows and gilts, respiratory disease in young growing pigs, and increased mortality in pre-weaning pigs (2, 6).

Antigenic variation among PRRSV isolates was first demonstrated between North American and European PRRSV isolates by the immunoperoxidase monolayer assay using PRRSV specific polyclonal antibodies (1, 7). Similar antigenic variation between European and North American isolates has also been shown by the indirect fluorescent antibody (IFA) test using three different monoclonal antibodies specific for the 15 kD nucleocapsid protein (3, 5).

While these earlier studies demonstrated that antigenic differences exist between European and North American PRRSV isolates, they failed to demonstrate significant antigenic variation among North American isolates. This

led to the routine use of a single Mab for the diagnosis of PRRV in clinical specimens. Subsequently work in this laboratory suggested that North American PRRSV isolates were more antigenically diversified than previously believed and that the antibodies currently in use for diagnostic purposes did not detect all field isolates (8). These observations led to the following study in which monoclonal antibodies were developed that could be used for the detection of PRRSV in clinical specimens with a higher degree of reliability.

Materials and Methods

Virus

A total of 67 PRRSV field isolates representing 13 states, Canada, the European Lelystad virus and the current North American vaccine strain and ATCC VR-2332 also known as ISU-P were used in the study. The field strains were derived from clinical specimens submitted to the National Veterinary Services Laboratories, USDA/APHIS/VS, Ames, Iowa, the Rollins Animal Disease Laboratory, Raleigh, North Carolina and the Iowa State Veterinary Diagnostic Laboratory, Ames, IA during 1989 through 1996.

Isolate ISU-P was used to produce antigen for mouse immunization in the production of Mab, and for the IFA test. This isolate was obtained from a swine herd in the state of Illinois with clinical PRRS

Production of monoclonal antibody specific for PRRSV

Murine monoclonal antibodies were produced using a standard protocol. Antibody producing hybridoma cells were identified by screening cell supernatant with an enzyme-linked immunosorbent assay and a FA test using MA104 cells infected with isolate ISU-P as antigen. Antibody-secreting hybridomas were cloned twice by a cell sorter. Classes and subclasses of individual MABs were determined using a mouse monoclonal antibody isotyping kit (Boehringer Mannheim®). The specificity of Mab's was determined by Western immunoblotting. A standard serum virus neutralizing assay was used to determine if Mab's were capable of neutralizing virus.

Categorization of monoclonal antibodies and field isolates

All 67 PRRSV isolates were tested in duplicate for reactivity with each Mab in the IFA on three separate days. The patterns of reactivity were then used to categorize both virus isolates and Mab's into distinct groups.

Results and Discussion

All of 22 MABs generated were IgG1 and specific for the 15 kD protein of PRRSV. No Mab had neutralizing

activity against PRRSV isolate ISU-P, the isolate used in producing the Mab's.

The categorization of Mabs and PRRSV isolates into specific groups is summarized in Table 1. Seven distinct groups (A-G) of Mabs were identified based on their reactivity with different PRRSV isolates in the IFA test. Group A Mabs reacted with all of the 67 isolates tested which suggests that these Mabs can be used as universal diagnostic Mabs. In contrast the European Lelystad virus, Group VII, is recognized only by Group A and Group D Mab.

The 67 PRRSV field isolates were categorized into seven groups (I-VII). Fifty six of the 67 isolates (83.6%) belong to Group I. This group of viruses was recognized by all Mabs tested. The ATCC VR-2332, prototype vaccine strain and the ISU-P isolate are also included in this Group.

The number of viruses represented by the remaining Groups range from one to four. These observations demonstrate that North American PRRSV isolates are antigenically more diverse than previously believed.

References

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Table 1. The reactivity of 22 monoclonal antibodies to 67 PRRSV isolates in indirect fluorescent antibody test.

| PRRS virus | | Group of Monoclonal antibodies | | | | | | |
|------------|----------------|--------------------------------|-----|----|----|----|----|-----|
| Group | No. of isolate | A | B | C | D | E | F | G |
| I | 56* | + | + | + | + | + | + | + |
| II | 1 | + | + | + | + | + | + | - |
| III | 1 | + | + | + | + | + | - | + |
| IV | 2 | + | + | + | - | - | + | + |
| V | 4 | + | + | - | - | - | + | + |
| VI | 2 | + | + | - | - | + | - | + |
| VII | 1 | + | - | - | + | - | - | - |
| | 67 | B5 [‡] | A23 | G6 | J2 | H1 | G9 | D12 |

*number of PRRS virus isolates categorized to antigenic group

[‡]representative monoclonal antibody of group