

Differentiation of F38 Mycoplasmas Causing Contagious Caprine Pleuropneumonia with a Growth-Inhibiting Monoclonal Antibody

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Monoclonal antibody WM-25 inhibited the in vitro growth of 13 F38 isolates from goats with contagious caprine pleuropneumonia but not 7 heterologous mycoplasma isolates representing four different species. In contrast to results with polyclonal antisera, growth inhibition by monoclonal antibody WM-25 was specific for F38 mycoplasma isolates and constituted a reliable means of distinguishing F38 from other mycoplasmas.

Contagious caprine pleuropneumonia (CCPP) is a disease of major economic importance in Asia and Africa, posing a major constraint to goat production because of high mortalities. The disease occurs in 33 countries; 8 others are on a suspect list (2). Mycoplasma F38, isolated from a goat with pleuropneumonia in Kenya (16), produces classical CCPP, which includes transmission by contact, high mortality, and a fibrinous pleuropneumonia characterized by massive hepatization and pleurisy. Similar mycoplasmas are isolated from goats in Sudan (11) and Yemen (J. K. Bari, M.S. thesis, Washington State University, Pullman, 1984). There is a need to establish whether F38 causes the CCPP reported in other countries (2).

Initial studies of F38 from Kenya suggested it might represent a new mycoplasma species (7). Also, electrophoretic analysis showed that F38 had isoenzyme patterns distinct from those of other mycoplasmas (22). However, in serological tests, F38 cross-reacts with several already established mycoplasma species (6-8, 13, 14). Further work using DNA hybridization (3) and two-dimensional electrophoresis of cell proteins (1, 19) did not clearly distinguish F38 from other mycoplasmas isolated from goats. Growth inhibition (4) and indirect epifluorescence (5) assays are considered species specific and are used to classify mycoplasma isolates (9). In these later tests, F38 cross-reacts with two other mycoplasmas which do not cause CCPP, bovine serogroup 7 and *Mycoplasma capricolum* (14, 15). This communication describes a monoclonal antibody which differentiates F38 by growth inhibition from other mycoplasmas.

To make monoclonal antibodies to F38, five BALB/c mice were injected intraperitoneally with 0.2 ml of a homogenized mixture of F38 organisms (0.2 mg of protein) and Freund complete adjuvant. The mice received two additional injections, 2 weeks apart, except with Freund incomplete adjuvant. The mice sera were tested for antibody activity against F38 antigen by an indirect enzyme-linked immunosorbent assay (ELISA) (20). Micro-ELISA plates with flat-bottom wells (Immulon, Dynatech-Deutschland Ambtt, 7310 Plochingen, Federal Republic of Germany) were coated with 100 μ l containing 100 μ g of F38 protein suspended in 0.1 M carbonate buffer, pH 9.0, with 0.0025% glutaraldehyde, at

37°C for 18 h. Plates were washed three times with 0.01 M phosphate-buffered saline, pH 7.2, containing 0.1% Tween 20. Culture supernatants (200 μ l) were added to the coated wells in duplicate, incubated at 37°C for 1 h, and washed three times. Antibodies to mouse serum conjugated to horseradish peroxidase were added, reacted at 37°C for 1 h, and the plates were washed. Substrate, 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)-diammonium salt (100 μ l), was added for 30 min at room temperature and in the dark. The color change was read at 405 nm by a micro-ELISA reader. The mouse with the highest titer of antibody was inoculated intravenously with F38 antigen. Four days later, the mouse was killed and spleen cells were prepared for fusion (18). Briefly, Sp2/O myeloma cells in logarithmic growth were fused with the spleen cells in the presence of 50% polyethylene glycol 1500. Specific antibodies were detected in culture supernatants by ELISA, and those found positive were tested for F38 growth-inhibiting activity (4). Cells producing a growth-inhibiting antibody were cloned twice by limiting dilution, and the isotype was determined by immunodiffusion against rabbit antisera to mouse immunoglobulin isotypes (Litton Bionetics, Kensington, Md.).

Monoclonal antibody WM-25, an immunoglobulin M which caused growth inhibition of F38, was isolated by gel filtration (21) from the ascitic fluid of pristane-primed BALB/c mice given cloned WM-25-producing cells intraperitoneally. Fifty microliters of the isolated WM-25 (2.2 mg/ml) was tested for growth inhibition of 13 mycoplasmas isolated from goats dying of CCPP (10) and identified as F38 by growth inhibition with a polyclonal antiserum to F38 (Table 1). The F38 isolates included prototype F38 (16), G92/81, G121/83, G152-4/83, G175/85, G183/82, G146/83, G94/83, G275/82, 232/82, G141/84, A/Narok/81, 2584, and G109/83. All these isolates were from different areas of Kenya except for G109/83, which was isolated from a goat lung from the Yemen Republic. All 13 isolates were inhibited by WM-25 but not by an unrelated immunoglobulin M control monoclonal antibody (TPM-12). In contrast, WM-25 did not inhibit growth of seven other mycoplasmas tested, even at mycoplasma concentrations 10^2 to 10^3 less than the concentration used for F38 isolates (Table 1). Included were two mycoplasma species previously shown to cross-react with F38, namely *M. capricolum* (3) and bovine serogroup 7 (14). Polyclonal antiserum to each of the seven mycoplasmas caused growth inhibition of the homologous mycoplasma.

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TABLE 1. Growth inhibition of F38 and other mycoplasmas with monoclonal and polyclonal antibodies to F38

Mycoplasma	Range of CFU/ml	No. of isolates with growth inhibition/no. tested		
		Monoclonal antibody WM-25	Control monoclonal antibody TPM-12	Polyclonal antiserum to F38
F38	1.3×10^7 – 1.5×10^9	13/13	0/13	13/13
Non-F38 ^a	0.3×10^5 – 2.5×10^5	0/7	0/7	1 ^b /7

^a The non-F38 mycoplasmas were *M. mycoides* subsp. *mycoides* Blenheim (small colony) and Y-goat (large colony), *M. mycoides* subsp. *capri* Smith 1423, *M. capricolum* (NCTC 10154), *M. equigenitalium* (NCTC 10176), *M. primatum* (NCTC 1163), and bovine serogroup 7 (NCTC 10133). The growth of these mycoplasmas was inhibited by the homologous antiserum. The source and classification of these mycoplasmas was described previously (12).

^b The non-F38 mycoplasma neutralized by polyclonal antiserum to F38 was bovine serogroup 7 (NCTC 10133).

Polyclonal antiserum to F38 caused growth inhibition of only one of the other mycoplasmas tested, bovine serogroup 7 (Table 1). Polyclonal antiserum to bovine serogroup 7 caused growth inhibition of prototype F38 (data not shown). Other workers have shown that polyclonal antiserum to *M. capricolum* causes growth inhibition of F38 and growth inhibition of *M. capricolum* occurs with polyclonal antiserum to F38 (14). Differences in the polyclonal antiserum could explain why this later cross-reaction by growth inhibition with polyclonal antisera was not seen in this study.

The mechanisms by which antibody inhibits the growth of mycoplasmas are not clearly understood. The fact that monoclonal antibody WM-25 did not inhibit the growth of heterologous mycoplasmas would suggest that these mycoplasma species do not possess the epitope recognized by WM-25 or that, if they do, the epitope either is not exposed on the live mycoplasma or has changed to the extent that WM-25 binding does not inhibit growth.

In conclusion, monoclonal antibody WM-25 caused growth inhibition of 13 F38 mycoplasma isolates and did not inhibit 7 other mycoplasma species. Use of WM-25 in growth inhibition resolved the only cross-reaction observed in this study, i.e., cross-growth inhibition of F38 and bovine serogroup 7 with polyclonal antisera. These data suggest that growth inhibition by WM-25 could be used to identify and classify F38 mycoplasmas.

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