**Monoclonal antibodies to a virulent strain
of transmissible gastroenteritis virus:
comparison of reactivity with virulent and attenuated virus**

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Summary. Twelve hybridomas secreting monoclonal antibodies (MAbs) against Miller virulent strain of transmissible gastroenteritis virus (TGEV) were generated and characterized. In a cell culture immunofluorescence (CCIF) assay, three MAbs directed against peplomer protein (E 2) had perinuclear fluorescence and four unclassified MAbs showed cell membrane fluorescence. Six of these seven MAbs neutralized both attenuated and virulent TGEV, and the seventh (an unclassified MAb) neutralized only the latter virus. Two MAbs able to bind the cell membrane of infected cells had low neutralizing antibody titers (8 to 72) but were able to distinguish between virulent and attenuated TGEV (9- to 72-fold differences in neutralizing titers). Two E 2-specific MAbs had higher neutralizing antibody titers (782 to 34,117) and showed 4- to 13-fold differences in titers against the attenuated and virulent TGEV strains. Five MAbs which were specific for nucleocapsid (N) protein had cytoplasmic, particulate fluorescence in CCIF, and did not neutralize TGEV. Comparison of CCIF antibody titers of MAbs to the virulent and attenuated strains of TGEV indicated that differences existed in titers of most E 2 and all N-specific MAbs, with titers consistently higher against virulent TGEV (homologous strain).

Hyperimmune antisera prepared in gnotobiotic pigs against the attenuated, virulent and a recent isolate of TGEV immunoprecipitated the 3 major structural proteins of both the attenuated and virulent TGEV strains. Relative mol. wt. differences in the E 1 and E 2 proteins between the two virus strains were revealed using either the hyperimmune pig sera or MAbs. In addition to the 48 K N protein, a 44 K protein was coimmunoprecipitated by the hyperimmune sera and MAbs, but mainly from lysates of attenuated TGEV.

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Introduction

Transmissible gastroenteritis virus (TGEV) belongs to the genus coronavirus of the family Coronaviridae [18]. It causes enteric disease in swine, producing a usually fatal diarrhea in seronegative piglets less than 2 weeks old [18]. Although there is only one known serotype, both attenuated and virulent strains of TGEV have been described [1]. Despite development of inactivated or live attenuated vaccines, no safe, effective, practical prophylaxis is yet available [18]. Thus a need exists to further define the protective antigens of TGEV and to evaluate possible antigenic differences between attenuated and virulent strains of TGEV.

Transmissible gastroenteritis virus possesses club-shaped peplomers and is enveloped and pleomorphic, with a diameter of 60–160 nm [16]. Analysis of TGEV revealed three major structural and two minor nonstructural proteins [5]. The glycosylated peplomer protein (E2) has an apparent molecular weight (mol. wt.) of 160–200 K and elicits neutralizing antibodies [16]. The nucleocapsid protein (N), associated with the RNA genome, is phosphorylated and has a mol. wt. of 50–56 K [5]. The matrix or transmembranous protein [E1] is also glycosylated and has a mol. wt. of 25–33 K [5]. The two minor proteins whose functions are unknown have mol. wts. of 105 K and 80.5 K. Recently, a 17 K intracellular protein was identified in cells infected with the Purdue strain of TGEV [21]; its function is also unknown.

In two previous studies, monoclonal antibodies (MAbs) to the structural proteins of the attenuated (Purdue) strain of TGEV were described [9, 12]. There are no published reports describing MAbs to a virulent strain of TGEV. In the present study, a panel of MAbs specific for structural proteins of the virulent (Miller) strain of TGEV was produced. These MAbs were further characterized in various comparative assays including cell culture immunofluorescence, virus neutralization, and radioimmunoprecipitation for reactivity against the Miller virulent and Purdue attenuated strains of TGEV.

Materials and methods

Cell culture

Primary porcine kidney (PPK) cells were used for propagation of TGEV and a swine testicle (ST) cell line was used in various assays (described below). Both PPK and ST cells were maintained in Eagle's Minimum Essential Medium (MEM) (GIBCO, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS) (GIBCO) and 100 µg/ml of gentamicin (Schering Veterinary, Kenilworth, NY).

Virus strains

For virus neutralization (VN), cell culture immunofluorescence (CCIF), and radioimmunoprecipitation assays, the Purdue attenuated strain (P115) and low cell culture-passaged Miller 6 (M6) virulent strain of TGEV were used. Both virus stocks were prepared in PPK cells. Viruses were harvested 48 h post-infection by three cycles of freezing and thawing, and the viruses stored in aliquots at -70°C . The Miller virulent strain (M5C) of TGEV

has been maintained by five serial passages in gnotobiotic pigs and represents the reference challenge strain of TGEV [18]. The field Zy isolate of TGEV was obtained during an outbreak of TGEV in 1986 from a 7 day-old diarrheic pig. Both the M 5 C and M 6 strains of TGEV, and the recent field Zy isolate of TGEV produced clinical signs and lesions typical of virulent TGEV in gnotobiotic pigs, including vomiting, diarrhea and villous atrophy. Pools of intestinal contents from M 5 C infected gnotobiotic piglets were purified as described in the following section.

Virus purification

Intestinal contents containing M 5 C TGEV or TGEV negative intestinal contents (from noninfected gnotobiotic pigs) were diluted 1 : 2 in Tris-CaCl₂ buffer 0.05 M Tris-HCl, 0.1 M NaCl, and 1 mM CaCl₂, pH 7.5 and sonicated for 1 min (BIOSONIK III, Bronwill, Rochester, NY) on ice. Crude suspensions were clarified by low speed centrifugation at $7,700 \times g$ for 30 min and supernatant fluids were layered onto discontinuous sucrose gradients of 20%, 35%, and 50% in Tris-CaCl₂ buffer. After centrifugation at $107,000 \times g$ for 3 h, the light-scattering bands at 20%/35% and 35%/50% interphases were collected separately and diluted 1 : 2 in Tris-CaCl₂ buffer. Sucrose was removed by pelleting virus at $135,000 \times g$ for 2 h and virus pellets were resuspended in Tris-CaCl₂ buffer. For each fraction, virus integrity was assessed by immune electron microscopy (IEM) [19] and virus titers were determined by CCIF (reciprocal of the endpoint dilution showing immunofluorescing cells). The fractions (20%/35% and 35%/50% interphases) with intact viral particles and virus titers $> 10^5$ were used to immunize BALB/c mice.

Cell culture immunofluorescence (CCIF) test

Confluent ST cell monolayers in 96-well plates were infected with either M 6 or P 115 strains of TGEV at a multiplicity of infection (m.o.i.) of 0.02 PFU/cell in Eagle's MEM. After incubation at 37 °C for 18 h, monolayers were rinsed with phosphate buffered saline (PBS), pH 7.4 and fixed with 80% acetone. The fixed cells were used for CCIF immediately. One hundred microliter per well of undiluted cell culture fluids from fusion plates or from limiting dilution plates or serial two-fold dilutions of ascites were added. The plates were incubated at 37 °C in a humid incubator for 1 h, and then rinsed with PBS for 10 minutes. Goat anti-mouse IgG + IgA + IgM conjugated to fluorescein isothiocyanate (FITC) (Kirkgaard & Perry, Gaithersburg, MA) at a 1 : 30 dilution was added to each well. After 1 h incubation at 37 °C, plates were rinsed once with PBS, pH 7.4 and once with PBS, pH 8.0 for 10 min. Following addition of a drop of mounting medium (60% glycerol in PBS, pH 8.0), cells were examined for immunofluorescence (indicative of the presence of TGEV antibodies) using a fluorescence microscope (Olympus IM, Japan).

Virus neutralization

To screen hybridomas for virus neutralizing (VN) antibodies to TGEV, a cytopathic effect (CPE) reduction assay was performed. Briefly, 100 μ l of hybridoma cell culture fluids were transferred to each well of a 96-well plate. An equal volume of 100–300 TCID₅₀/100 μ l of M 6 or P 115 strain of TGEV was added. The mixture was incubated at 37 °C for 1 h and 50 μ l of 10^6 cells/ml of an ST cell suspension in Eagle's MEM supplemented with 10% FBS was added to each well. The plates were incubated at 37 °C for 48 h and neutralizing activity was determined by the absence of CPE.

A plaque reduction assay was performed using 7-day old ST cell monolayers in six-well plates to determine virus neutralizing antibody titers. Equal volumes of M 6 or P 115 strains of TGEV containing 50 to 60 plaque forming units (PFU) in 100 μ l were added to serial fourfold dilutions of heat inactivated (56 °C, 30 min) ascites fluids and incubated at

37°C for 1 h. Then, 100 µl of inoculum were added to duplicate wells followed by an additional 1 h incubation at 37°C. To each well, 4 ml of 0.8% noble agar and 0.7% (of 0.1% stock) neutral red in Eagle's MEM were added. The virus neutralizing antibody titers were expressed as the reciprocal of the highest sample dilution which produced an 80% reduction in plaques when compared to the virus control wells.

Production of hyperimmune porcine sera

Seronegative gnotobiotic pigs were used for production of TGEV hyperimmune sera. Two pigs were inoculated with M 5 C virulent TGEV, one pig with Zy isolate TGEV and 1 pig with P 115 attenuated TGEV. Each pig was inoculated orally with 2–5 ml of TGEV and subsequently hyperimmunized (both intramuscularly and subcutaneously), one time with virus mixed with an equal volume of Freund's complete adjuvant at two weeks post-oral inoculation, and three times with virus mixed with Freund's incomplete adjuvant, at weekly intervals. Sera were collected 7 days after the last injection.

Production of hybridomas

Hybridomas were produced using modifications of procedures described previously [10]. To obtain hybridomas secreting TGEV specific MAbs, female BALB/c mice were hyperimmunized five times at weekly intervals with semi-purified M 5 C strain of TGEV [approx. 10^6 fluorescent focus units (FFU)/ml]. Spleen cells from these mice were fused with SP 2/0 myeloma cells at a ratio of 2:1 in the presence of 50% polyethylene glycol (MW 3350, Sigma, St. Louis, MO).

Hybridomas secreting MAbs to TGEV were detected by VN and CCIF tests two times at 7 day intervals. Selected clones which were positive by either test were subcloned at least two times by limiting dilution [15] using conditioned medium which was prepared as follows: thymocytes and spleen cells from female BALB/c mice were prepared using 2×10^5 cells/ml and maintained in RPMI 1640 supplemented with 20% FBS at 37°C for 5 days. The supernatant medium was then removed and stored at 4°C for use in limiting dilution.

Determination of isotype and subisotype

The isotype and subisotype of MAbs were determined by using the Ouchterlony immunodiffusion technique [17]. Monoclonal antibodies produced in culture medium were precipitated with 50% ammonium sulfate and resuspended to $10 \times$ the initial concentration. Monospecific antiserum to each isotype or subisotype was purchased commercially (ICN Immunobiologicals, Lisle, IL).

Production of ascites

Ascites fluids containing MAbs were produced in pristane (2,6,10,14-tetramethyl pentadecane, Aldrich Chemical Co., Milwaukee, WI) primed-mice as described previously [15]. Mice were primed at least 7 to 10 days prior to use. Approximately 1 to 2×10^6 hybridoma cells were injected intraperitoneally. Ascites was produced and the fluids harvested 10–14 days post-injection.

Radiolabeling and radioimmunoprecipitation (RIP)

Tests were performed using modifications of procedures described by Wesley and Woods [21]. Briefly, seven-day old confluent ST cell monolayers grown in 75 cm² flasks were infected with M 6 TGEV at an m.o.i. of 0.04 PFU/cell or P 115 TGEV at an m.o.i. of 0.02 PFU/cell. Mock infections using tissue culture media were done concurrently for each virus. At 7 h post inoculation (PI) mock and TGEV infected monolayers were washed with and

maintained in methionine-deprived Eagle's MEM supplemented with 100 µg/ml of gentamicin. Labeling experiments were repeated using 1 µg/ml of actinomycin D added at 7 h PI for studying the effect of actinomycin D on viral protein synthesis and for reducing the amount of labeled host cell proteins. At 8 h, PI, 100 µCi/ml of L-³⁵S-methionine (Amersham, Arlington Heights, IL) was added to each flask containing 3 ml of methionine-deprived medium. The flasks were incubated at 37 °C for an additional 3 h with gentle agitation. The flasks were then rinsed with PBS (pH 7.4) containing 1 mM phenylmethylsulphonyl fluoride (PMSF, Sigma), and the cell monolayers were lysed in 3 ml lysis buffer 0.15 M NaCl, 0.6 M KCl, 0.5 mM MgCl₂, 10 mM Tris-HCl, pH 7.8, 2% Triton X-100, and 10³ units/ml Aprotinin). Cells were lysed at room temperature for 30 min and cell debris and particulate material were removed by centrifugation at 135,000 × g for 30 min. The supernatant was stored in 400 µl aliquots at -80 °C.

The RIP assay was performed using modifications of methods described by Wesley and Woods [21]. Cell lysates were preabsorbed with pooled normal mouse sera bound onto protein-A Sepharose (Pharmacia, Sweden) at 37 °C for 1 h. Undiluted mouse ascites fluids (40 µl) or hyperimmune porcine sera (10 µl) were absorbed onto protein-A Sepharose and then reacted with unlabeled, uninfected ST cell lysate for 1 h at 37 °C. This treated ascites or sera bound to protein-A Sepharose was mixed with 100 to 200 µl of preabsorbed cell lysate at 37 °C for 1 h and then at 4 °C overnight. The Sepharose was pelleted in a microfuge and washed four times with lysis buffer and one time with 0.05 M Tris-HCl (pH 8.0) buffer. The immune complexes bound to Sepharose were pelleted and 100 µl of Laemmli's sample buffer [11] was added. The mixtures were heated at 95 °C for 5 min and the Sepharose was pelleted in a microfuge. The resulting supernatants were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in 5% stacking and 10% (100: 1-acrylamide: bis) running gels. The gels were treated with EN³HANCER (New England Nuclear, Boston, MA), dried and exposed to X-ray films (Kodak XRP-5) at -80 °C for 2 days [2]. Molecular weight standards used were ¹⁴C-methylated myosin (200,000), phosphorylase-b (92,500), bovine serum albumin (69,000), ovalbumin (46,000), carbonic anhydrase (30,000), and lysozyme (14,300) (Amersham). Negative controls including a MAb against OSU porcine rotavirus, SP 2/0 cell-induced ascites, and TGE negative porcine and mouse sera were also analysed by RIP.

Results

A panel of twelve MAbs against the virulent strain of TGEV was generated and their reactivities against virulent and attenuated TGEV characterized (Table 1). Their isotype and subsotype specificity was also determined (Table 1).

Protein specificity

Protein profiles of P 115 and M 6 infected cell lysates are shown in Fig. 1. Viral specific proteins (E 1, E 2, and N) were evident for both P 115 and M 6 infected cell lysates. No differences in protein profiles were observed with or without the presence of actinomycin D (data not shown). A unique approx. 14 K species of protein was revealed only in the P 115 infected cell lysates (Fig. 1).

Seven neutralizing and five non-neutralizing MAbs were selected for characterization of their viral protein specificity by RIP of ³⁵S-methionine labeled P 115 and M 6 TGEV infected cell lysates (Table 1). A faint band in the 30 K (E 1) region was evident with MAbs 25 D 11, 25 A 4, 1 H 8, and 13 D 8 (in Fig. 2A represented by 25 D 11), but because of the low intensity of this reaction and

Table 1. Characteristics of monoclonal antibodies produced against the Miller virulent strain of TGEV

Hybridoma	Ig Isotype	Geometric mean neutralizing antibody titer (range) against ^a		CCIF antibody titer against		Immunofluorescence pattern	Protein specificity Immunoprecipitation
		P 115	M 6	P 115	M 6		
1H8	G ₁	<1	72 (64-80)	8,000	8,000	Cell membrane	ND ^b
13D8	G ₁	8 (3-18)	70 (62-78)	2,000	2,000	Cell membrane	ND
25A4	G ₁	234 (180-305)	338 (300-380)	16,000	25,000	Cell membrane	ND
25D11	G _{2a}	170 (78-370)	320 (270-380)	8,000	8,000	Cell membrane	ND
25H4	G _{2a}	34,117 (9,650-56,000)	8,253 (1,250-18,500)	6,400	51,200	Diffuse perinuclear	E 2
25C9	G _{2a}	70,268 (25,000-90,000)	34,672 (23,000-48,000)	12,800	51,200	Diffuse perinuclear	E 2
25E4	G _{2a}	782 (250-4,096)	9,795 (6,400-17,000)	12,800	51,200	Diffuse perinuclear	E 2
14E3	G ₁	<1	<1	12,800	102,400	Cytoplasmic particulate	N
14F10	G ^c	<1	<1	12,800	102,400	Cytoplasmic particulate	N
14G9	G ₁	<1	<1	25,600	102,400	Cytoplasmic particulate	N
24A11	G _{2b}	<1	<1	6,400	25,600	Cytoplasmic particulate	N
25H7	G _{2a}	<1	<1	6,400	102,400	Cytoplasmic particulate	N

^a Neutralizing antibody titer expressed as the reciprocal of the ascites dilution which produced 80% plaque reduction compared to controls.

Numbers represent geometric mean titer (3 or 4 replicates) and titer ranges (parentheses)

P 115 attenuated TGEV; M 6 virulent TGEV

^b ND Not determined^c Subisotype could not be determined

Fig. 1. Protein profile of ^{35}S -methionine labeled TGEV infected ST cell lysates and mock infected ST cell lysate controls at 11 h post-infection and 3 h post-labelling. *C*₁ Mock infected ST cell lysate for P 115 virus; *P* P 115 TGEV infected ST cell lysate; *C*₂ mock infected ST cell lysate for M 6 virus (in the presence of 1 $\mu\text{g}/\text{ml}$ actinomycin D); *M* M 6 TGEV infected ST cell lysate (in the presence of 1 $\mu\text{g}/\text{ml}$ actinomycin D). Molecular weight markers are shown in the far right lane. Position of viral proteins (E 2, N, E 1, 14 K) are identified by large arrowheads. *O* Origin, *BB* bromophenol blue dye marker, *E* 1 membrane protein, *E* 2 peplomer protein, *N* nucleocapsid protein

the high backgrounds observed, the protein specificity of these four MAbs was considered inconclusive and they were labeled as unclassified. Monoclonal antibodies 25 H 4, 25 C 9 and 25 E 4 immunoprecipitated 200–220 K E 2 proteins (Table 1; in Fig. 2B represented by 25 H 4). The relative mol. wt. of the E 2 proteins from M 6 immunoprecipitates was higher than for E 2 proteins from P 115 immunoprecipitates. Monoclonal antibodies 14 E 3, 14 F 10, 14 G 9, 24 A 11, and 25 H 7 immunoprecipitated 44 K and/or 48 K N proteins (Table 1; in Fig. 2C represented by 25 H 7). The 44 K protein band was consistently more intense in immunoprecipitates of the P 115 than of the M 6 strain of TGEV. A

Fig. 2. Protein specificity determined by radioimmunoprecipitation (RIP) of 3 representative monoclonal antibodies reactive with each of the TGEV major structural proteins tentative, E 1 (A), E 2 (B), and N (C). 35 S-methionine labeled cell lysates of TGEV infected cultures or mock infected cultures were reacted with undiluted MAb (25 D 11, 25 H 4, and 25 H 7) and processed for RIP. Resulting immune complexes were analysed on 10% SDS-polyacrylamide gels and the gel autoradiographs were exposed for 2 days. *P* P 115 infected lysate; *M* M 6 infected lysate; *C* mock infected control. Molecular weight markers are shown in the far left lane. *O* Origin, *BB* bromophenol blue dye marker, *E* 1 membrane protein, *E* 2 peplomer protein, *N* nucleocapsid protein

band (possibly actin) with a mol. wt. of 46 K as consistently resolved in immunoprecipitation of mock and TGEV infected cell lysates (Fig. 2A, B, C). TGE viral specific proteins (E 1, E 2, and N) were not immunoprecipitated by negative control sera or ascites fluid (e.g., MAbs against porcine OSU rotavirus, SP 2/0 cells induced ascites fluid and TGEV negative mouse and porcine sera) (data not shown).

Results of RIP of P 115 or M 6 TGE viral proteins using the four hyper-immune sera are shown in Fig. 3. The viral proteins immunoprecipitated by these sera were compared with those recognized by the MAbs. The three major TGE viral proteins (E 1, E 2, N) from M 6 or P 115 infected cell lysates were immunoprecipitated by each of the hyperimmune sera prepared against the three TGE strains (virulent, attenuated and field isolate). The relative mol. wt. of the E 2 protein was consistently higher and the mol. wt. of the E 1 protein was consistently lower for the M 6 strain of TGEV than for P 115 TGEV. Sera

Fig. 3. Immunoprecipitation of TGE viral proteins using 4 hyperimmune sera: *anti-M5C* virulent TGEV; *anti-Zy* field isolate TGEV; and *anti-P115* attenuated TGEV serum. *M* M6 infected ST cell lysate; *P* P115 infected ST cell lysate; *C* Mock infected ST cell lysate. *O* origin, *BB* bromophenol blue dye marker, *E1* membrane protein, *E2* peplomer protein, *N* nucleocapsid protein

produced against all three strains of TGEV immunoprecipitated the 44 K protein from P115 infected cell lysates: this viral protein was undetectable or of low intensity in lysates from M6-infected cells (Fig. 3).

Virus neutralizing reactivity of MAbs with attenuated and virulent strains of TGEV

Seven of the selected hybridomas produced TGE virus neutralizing antibodies (three E2-specific and four unclassified MAbs) (Table 1). Four to 72-fold differences in neutralizing antibody titers against attenuated and virulent TGEV were observed for unclassified MAbs 1H8 and 13D8, and 25H4 and E2-specific MAb 25E4 (Table 1). Monoclonal antibody 1H8 did not neutralize the P115 attenuated strain of TGEV (titer <1); whereas it has a titer of 72 against the M6 virulent strain of TGEV. Monoclonal antibodies 13D8 and 25E4 (anti-E2) had 9 to 13-fold higher neutralizing antibody titers against M6 than against the P115 strain of TGE virus. Both P115 and M6 TGEV produced

plaques larger than those in virus control wells (data not shown) in the presence of MAb 1 H 8 and 13 D 8 in plaque reduction assays. Only MAbs 25 H 4 and 25 C 9 had higher neutralizing antibody titers (2 to 4-fold) against P 115 than against M 6 TGEV (Table 1). None of the anti-N MAbs neutralized TGEV.

Immunofluorescence staining patterns and reactivity of MAbs with attenuated and virulent strains of TGEV

All twelve MAbs reacted with TGEV in a CCIF test and five hybridomas produced TGEV antibodies which were detected only by CCIF. The antibody titers determined by CCIF against both P 115 and M 6 TGEV are summarized in Table 1. Using CCIF, four MAbs whose protein specificity was undetermined, showed cell membrane fluorescence (Fig. 4a). Three MAbs which reacted with E 2 protein showed faint diffuse perinuclear fluorescence (Fig. 4b). Those MAbs which reacted with N protein produced bright particulate cytoplasmic fluorescence (Fig. 4c). All MAbs except the four unclassified MAbs had higher CCIF titers against homologous (M 6) TGEV than heterologous (P 115) TGEV; 8-fold differences were observed for MAbs 25 H 4, 14 E 3, and 14 F 10 (anti-E 2 and N, respectively) and 16-fold differences were noted for MAb 25 H 7 (anti-N).

No differences in fluorescence staining patterns of MAbs directed against the same proteins were observed when either P 115 or M 6 was used to infect ST cells. However differences in fluorescence intensity were observed using either P 115 or M 6 as the test antigen (data not shown). Monoclonal antibody 14 E 3 and 14 F 10 produced stronger immunofluorescence with M 6 infected ST cells than P 115 infected ST cells at all dilutions tested. Monoclonal antibody 14 G 9, 25 A 11, and 25 H 7 at lower dilutions (1 : 100 to 1 : 1,000) induced similar fluorescence intensity with either P 115 or M 6 infected ST cells, but brightness diminished after 1 : 1,600 to 1 : 6,400 dilutions for P 115 infected ST cells.

Discussion

Monoclonal antibodies against the virulent strain of TGEV were generated, and viral protein specificities were determined for all but four MAbs. Anti-E 2 MAbs recognized E 2 proteins with mol. wt. of 200–220 K in RIP, as reported similarly by others for MAbs to P 115 TGE [12, 9]. Anti-N MAbs immunoprecipitated a 48 K protein derived from both P 115 and M 6 infected cell lysates, and a 44 K protein was co-immunoprecipitated mainly from P 115 infected cell lysates. The same finding was noted when three of the four hyperimmune porcine sera were tested. This unique 44 K protein has not been reported by other

Fig. 4. Immunofluorescence staining patterns of MAbs reacted with M 6 TGEV-infected ST cell monolayers. **a** Cell membrane IF observed with tentative anti-E 1 (1 H 8); **b** diffuse perinuclear IF observed with anti-E 2 (25 C 9); and **c** cytoplasmic particulate IF observed with anti-N (14 G 9)

Fig. 4

researchers. One explanation may be that the percentage of cross-linker in our gel system was different from others; therefore, the resolution for the 44 K protein was better. Our results suggest that although this 44 K protein occurs in both P 115 and M 6 infected cells and was detected by hyperimmune sera and MAbs produced against virulent TGEV, it accumulates more in the P 115 infected cells. The nature of this extra species recognized by N-specific MAbs is unclear. It may be a precursor or cleavage product of the viral N protein. More conclusive explanations may be obtained if the complete gene coding sequence for the N protein becomes available, or the kinetics for N protein synthesis are explored. In addition, a minor 14 K band detected in P 115 infected cell lysates, but not in M 6 cell lysates (11 hpi), may be similar to the 17 K protein described in P 115 cell lysates by Wesley and Woods [21] or to the 14 K protein described by Hu et al. [8]. Whether this 14 K protein is an intrinsic protein for only Purdue attenuated TGEV or a cleavage product of E 2 is not yet known. Nevertheless, this protein was not immunoprecipitated by the MAbs or the four hyperimmune swine sera.

The four MAbs which had low neutralizing antibody activity, showed high background and low intensity reactions in RIP and were reported as unclassified MAbs which may possibly be E 1-specific. Additional tests are required to confirm their protein specificities using either more extensive absorption methods or a solid phase immunoisolation technique [20].

Epitopes which elicited TGEV neutralizing antibodies resided on the E 2 and E 1 proteins [12]. In contrast, anti-E 1 MAbs produced by Jimenez et al. [19] did not neutralize TGEV. Anti-E 1 MAbs prepared against a mixture of virulent Miller 3 and Purdue attenuated strains of TGEV had virus neutralizing antibody titers only in the presence of guinea pig, rabbit or swine complement [22]. It is possible some anti-E 1 MAbs may inactivate TGEV infectivity via complement-mediated virolysis. Interestingly, all four unclassified MAbs (which may react with E 1 protein) produced in the present study had low to moderate virus neutralizing antibody titers, but the neutralizing activities were not enhanced in the presence of 4 hemolytic units/ml of swine complement (data not shown). By comparison, Woods et al. [22] reported that their E 1-specific TGEV MAbs had VN activity only in the presence of 2 to 8 hemolytic units/ml of swine complement.

The distinctive immunofluorescence patterns associated with the different TGEV protein specificities, suggest that a panel of such MAbs may be useful for studies of the morphogenesis of TGEV. Immunofluorescence patterns in TGEV-infected cells reacted with E 2-specific MAbs suggest that E 2 proteins are produced mainly in the cell perinuclear regions. Anti-N MAbs produced bright cytoplasmic particulate immunofluorescence in TGEV-infected ST cells suggesting that N proteins are abundant in cytoplasm and often in aggregates reflected by the particulate nature of the fluorescence. In the case of the four unclassified MAbs, fewer immunofluorescent TGEV-infected ST cells were evident (although all cells were infected with the same m.o.i.) and fluorescence

on the cell surface was more distinct around cell to cell junctions. Laude et al. [12] demonstrated 3 similar fluorescence patterns. However the membrane fluorescence was shown in paraformaldehyde-fixed cells reacted with an anti-E 2 MAb and a fluorescence pattern for anti-E 1 MAbs was not identified.

Biochemical and biophysical differences among field isolates and cell culture-attenuated TGEV have been reported [4, 7], but serologic differences were not identified [13, 14]. Using MAbs to Purdue TGEV in competitive assays, Delmas et al. [3] demonstrated that the neutralization mediating determinants were highly conserved among TGEV strains. Furthermore, Jimenez et al. [9] showed that neutralizing epitopes on attenuated TGE virus were conformational and highly conserved. Our data suggest differences exist between a virulent and attenuated strain of TGEV, perhaps in the density of certain epitopes expressed on the viruses, or the proteins they elicit in infected cells. First, neutralizing antibody titers of unclassified MAbs 1 H 8 and 13 D 8 and anti-E 2 MAb 25 E 4 were 72, 9 and 13-fold higher respectively, to M 6 than P 115 TGEV. Second, anti-E 2 MAb 25 H 4 had a 4-fold higher neutralizing antibody titer to P 115 than to M 6. Third, all anti-N and anti-E 2 MAbs had 4 to 16-fold higher CCIF titers to M 6 than P 115. Furthermore, fluorescence intensity was stronger against the M 6 strain than P 115 strain of TGEV. Finally, differences in the relative mol. wt. of E 1 and E 2 proteins and in the quantity of the 44 K protein between the virulent and attenuated TGEV strains, imply that phenotypic variation between the 2 strains may exist. This has not been reported previously.

Further studies are needed to define and compare TGEV epitopes on attenuated, virulent and field isolates of TGEV. Although major epitopes mediating neutralization were associated with E 2 proteins and these were conserved, nonneutralizing MAbs against E 1, E 2, or N proteins may be unique for certain isolates and could thus be used for differentiation of TGEV strains as shown previously by Laude et al. [12]. It is important to continue characterizing critical epitopes of the virulent strain of TGEV for future development of possible subunit or rDNA vaccines. Monoclonal antibodies to TGEV described in this study, as well as additional MAbs, should also aid in development of better diagnostic reagents for detection and possible differentiation of field and vaccine strains of TGEV.

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