ANTIBODY RESPONSE IN SWINE TO INDIVIDUAL TRANSMISSIBLE GASTROENTERITIS VIRUS (TGEV) PROTEINS

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INTRODUCTION

Transmissible gastroenteritis virus (TGEV) is a coronavirus that produces a life-threatening diarrhea in neonatal pigs. Whereas sows infected with virulent virus produce protective colostral antibody, sows vaccinated with attenuated virus are significantly less effective in passively protecting baby pigs. Therefore, an understanding of the antigenic and biochemical properties of pathogenic TGEV and the pigs' immune response to these antigens is a critical area of TGEV research. We are in the process of characterizing the porcine immune response to major TGEV antigens in a protein A immunoprecipitation assay. Protein A is known to bind approximately 90% of the swine immunoglobulin isotypes, IgG_1 and IgG_2, but is less efficient in binding either swine IgM or IgA.[1] This study has led to the serological identification of a new TGEV-specific low molecular weight polypeptide.

MATERIALS AND METHODS

Cells and Virus

Swine testicular (ST) cells were grown as described previously.[2] The Purdue strain of TGEV was propagated in ST cell cultures and the virulent Miller strain of TGEV, passed only in pigs, was used to infect pigs via the intranasal-oral route.

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Radioimmunoprecipitation Assay

At 10 hr. postinfection with TGEV, ST cell cultures were labeled for 2-4 hrs. with 100μCi/ml ^{35}S-methionine (NEN, 1084 Ci/mmol.). After washing 3 times with PBS - 1 mM phenylmethylsufonyl fluoride (PMSF) at 4°C, cell lysates were prepared at 45°C for 10 min. in NET buffer (150 mM NaCl, 5 mM EDTA, 50 mM Tris-HCl pH 7.5) containing 1% NP-40, 1% Na deoxycholate and 1 mM PMSF. Protein aggregates were removed by ultracentrifugation and aliquots of lysate were stored at -70°C. Uninfected ST cell mock lysates were similarly prepared as controls.

Radiolabeled extracts of TGEV-infected and mock infected cells (5 X 10^5 CPM) were twice preabsorbed with normal swine sera bound to Protein A Sepharose beads (Pharmacia). The extracts were then mixed with swine anti-TGEV sera bound to Protein A Sepharose, incubated for 1 hr. at room temperature and then overnight at 4°C. Bound immune complexes were washed 3 times in lysis buffer and twice in distilled water. Protein A bound immune complexes in the presence of sodium dodecyl sulfate (SDS) and 2-mercaptoethanol were denatured by boiling for 3 min. prior to SDS-PAGE electrophoresis on 12% gels. Amersham ^{14}C-labeled molecular weight marker proteins were included on each gel to allow molecular weight estimates of labeled proteins. Gels were fluorographed and exposed to preflashed Kodak X-ray film.

Step Sucrose Gradient Enrichment of the p14 Antigen

ST cell cultures as monolayers in stationary phase were infected with approximately 3 PFU/cell of TGEV. At 6 hr. postinfection, cells were labeled for 2 hr. with 25 μCi/ml ^{35}S-methionine and the cell-associated virus was partially purified as follows. Culture fluids were decanted, the infected cell monolayer rinsed in hypotonic solution (10 mM NaCl, 10 mM Tris-HCl pH 7.5, 1.5 mM $MgCl_2$) and 5 ml hypotonic solution per 75 cm^2 tissue culture flask was added for a freeze-thaw cycle. Swollen cells and debris were further disrupted with 10 strokes of a Dounce homogenizer, clarified, and the supernatant was pelleted through a 30% (w/w) sucrose cushion in TES buffer (20 mM Tris-HCl pH 7.4, 1 mM EDTA, 100 mM NaCl) in the Beckman SW 28 rotor at 25,000 RPM for 2 hr. at 4°C. The pellet was resuspended in 1.5 ml of TES buffer and used to overlay a step gradient consisting of 2.5 ml of each 45%, 40%, 30%, and 20% (w/w) sucrose in TES and centrifuged in the Beckman SW 41 at 26,000 RPM for 2 hr. at 4°C. The opaque bands at the 30/40 and 40/45 interfaces were pelleted by centrifugation in the SW 41 rotor, 30,000 RPM for 1 hr., 4°C, resuspended in TES buffer and an aliquot was denatured by boiling for 3 min. in 2X electrophoresis sample buffer containing 2-mercaptoethanol and analyzed on a 12% SDS-PAGE gel.

RESULTS

Radioimmunoprecipitation of TGEV Polypeptides by Swine Convalescent Sera

To investigate the antibody response to specific TGEV proteins in convalescent sera from swine exposed to virulent virus, cellular lysates of ST cells infected with the Purdue strain of TGEV were used as ^{35}S-methionine labeled antigens in a radioimmunoprecipitation assay. A hyperimmune pig antiserum prepared against the Miller strain of TGEV was used to mark the position of target antigens on the gel. With this hyperimmune antiserum, four distinct TGEV polypeptides are detected in ^{35}S-methionine labeled infected cell lysates. As shown in Figure 1, these polypeptides are the envelope glycoproteins, gp200 and gp29, the nucleocapsid protein p47 and a cell-associated polypeptide, p14. This latter TGEV-specific antigen previously was reported by us to have a molecular weight of 17 Kd.[2]

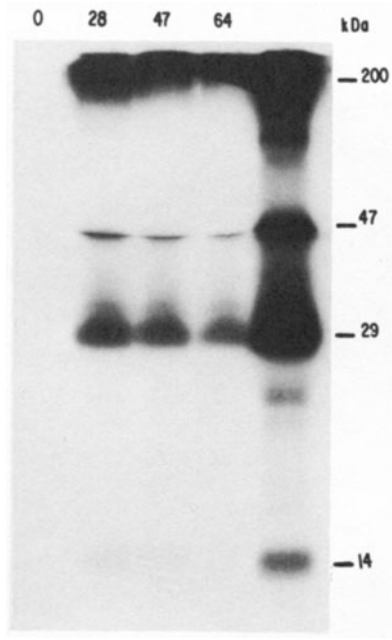

Fig. 1. Electrophoretically separated polypeptides immunoprecipitated from ^{35}S-methionine labeled, TGEV-infected cell extracts. Lanes 1-4, convalescent sera from a pregnant gilt taken at the time of infection (0) and at 28, 47, and 64 days postinfection. Lane 5, four TGEV antigens immunoprecipitated with a pig hyperimmune anti-TGEV serum.

Six feeder pigs and three pregnant dams (infected 6 weeks prior to farrowing) were infected with an intranasal-oral dose of virulent TGEV and convalescent serum samples, collected between 11 and 77 days postinfection, were assayed. Immunoprecipitation profiles monitoring predominantly the IgG antibody response were essentially the same for all pigs as exemplified by the pattern shown in Figure 1. The preinfection serum samples did not immunoprecipitate TGEV antigens. All postinfection serum samples reacted with the two surface viral glycoproteins gp200 and gp29. All but one pig showed an antibody response to the minor 14 Kd antigen. None of the convalescent serum samples from these older pigs reacted with the nucleocapsid protein, p47. The sharp bands in Figure 1 below the 47 Kd marker are nonspecific proteins in that these also are immunoprecipitated by convalescent sera in parallel uninfected control lysates. Antibodies to the viral surface glycoproteins were detectable in convalescent sera as early as postexposure day 11 and reached peak immunoprecipitation levels at 3 to 4 weeks post-TGEV infection. Serum antibody levels appeared to decline at different rates with the anti-gp29 and anti-p14 declining more rapidly than the anti-gp200 antibody activity.

Enrichment of the 14 Kd Antigen in the Cell-Associated Fraction

We have reported earlier that the low molecular weight polypeptide, p14, was absent in uninfected ST cell lysates but became apparent at 6 hr. postinfection.[2] Autoradiographs indicated that the 14 Kd polypeptide copurified as a minor component or contaminant with the cell-free virus in linear sucrose gradients. However, p14 mainly remained cell-associated and we have enriched for this polypeptide by pelleting cell-associated ^{35}S-labeled proteins through a sucrose step gradient. Figure 2 shows the results of hypotonic lysis of TGEV infected ^{35}S-labeled cells and centrifugation through a 20-30-40-45% (w/w) sucrose step gradient. Viral structural proteins from cell-associated virions were present at both the 30/40% and 40/45% sucrose interfaces and in the pellet but there was a distinct enrichment for the low molecular weight p14 antigen after pelleting through dense sucrose (ρ = 1.21 g/cc). This indicates that the 14 Kd polypeptide is associated with a rapidly-sedimenting, intracellular component that is more dense than TGEV.

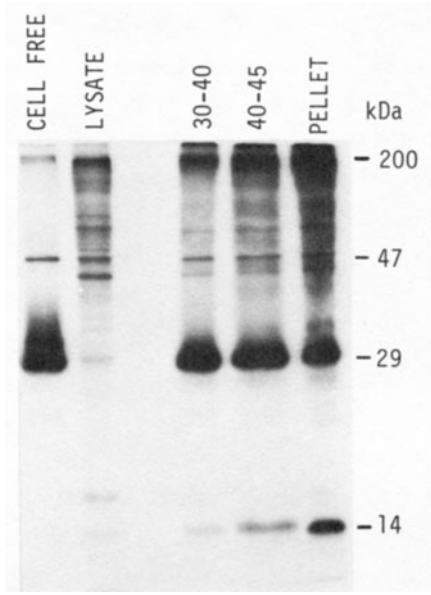

Fig. 2. SDS-PAGE electrophoresis of TGEV-infected cell extract and purified virus labeled with ^{35}S-methionine at 6-8 hr. postinfection. The two lanes on the left show protein profiles of purified TGEV and the intracellular proteins from the infected cell lysate. The right-hand three lanes indicate the enrichment of the cell-associated p14 polypeptide at the 30/40% and 40/45% interfaces, and the pellet of a sucrose step gradient.

DISCUSSION

Pregnant swine and feeder pigs (15-20 kg) were infected by the intranasal-oral route with the virulent Miller strain of TGEV and convalescent serum samples were analyzed by immunoprecipitation. Convalescent sera was most reactive at the end of the first month following a primary infection and responded to the viral peplomer protein, gp200, the matrix protein, gp29, and a low molecular weight TGEV-specific intracellular antigen, p14. Serum antibody directed against these three TGEV antigens, predominately IgG due to the protein A basis of the assay, declined at different rates with the anti-gp200 yielding the most prolonged response.

A TGEV-specific intracellular polypeptide of 14 Kd was shown to be a weak antigen by immunoprecipitation analysis. While some 14 Kd protein did copurify with the sucrose gradient purified cell-free virus, the majority of 14 Kd remained cell-associated and was enriched at densities in sucrose greater than for purified virus. Thus, the 14 Kd polypeptide appears to be associated with a cellular component of density greater than that for TGEV. Skinner and Siddell[3] have suggested that a murine hepatitis virus nonstructural protein of similar molecular weight might be associated with viral RNA in a replication complex. Our observations regarding the intracellular associations of the TGEV 14 Kd polypeptide with a dense, fast-sedimenting component are consistent with their hypothesis.

TGEV of pigs and feline infectious peritonitis virus (FIPV) of cats are antigenically related coronaviruses producing antibodies in their natural host that recognize common determinants on the three major virion structural proteins.[4] Each of these coronaviruses, however, produces a markedly different disease pathology in their host; a fatal enteric infection in neonatal swine (TGEV) or a progressively debilitating immunocomplex disease in cats (FIPV). Likewise, the serum antibody response to individual viral proteins is distinct for each disease. Following FIP infections, free antibody is directed primarily towards the nucleocapsid protein and the viral matrix glycoprotein whereas there is little reactivity to the FIP peplomer glycoprotein.[5,6] In contrast, we have shown by protein A-based immunoprecipitation that the major anti-TGEV response is to the two viral surface glycoproteins (gp 200 and gp 29) and that the anti-peplomer antibody is the most persistent following a primary TGEV infection.

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