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Induction of Alpha Interferon by Transmissible Gastroenteritis Coronavirus: Role of Transmembrane Glycoprotein E1

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Epithelial cells infected with the coronavirus transmissible gastroenteritis virus (TGEV) and fixed by glutaraldehyde induced a high alpha interferon (IFN-α) production in nonimmune porcine as well as human or bovine peripheral blood mononuclear cells (PBMC). IFN-α was detected as early as 3 h after exposure of PBMC to infected cells and at producer/inducer cell ratios as low as 1/1. Two of four monoclonal antibodies directed against the viral transmembrane glycoprotein E1 could block the IFN-inducing capacity of both TGEV-infected cells and viral particles. On the other hand, IFN-α induction was not markedly affected by monoclonal antibodies directed against other E1 epitopes, against peplomer glycoprotein E2, or against nucleocapsid protein. Thus, these findings strongly imply that IFN induction by TGEV results from interactions between an outer membrane domain of E1 and the PBMC membrane.

There is a limited number of reports showing that alpha interferon (IFN-α) can be induced after contact of leukocytes with noninfectious viral structures, such as viral envelopes, isolated viral glycoproteins, or virus-infected, glutaraldehyde-fixed cells. For instance, UV-inactivated Sendai virus (8), adenovirus fiber protein (20), and herpes simplex virus (HSV)- or dengue virus-infected glutaraldehyde-fixed cells (1, 9, 18, 19) were shown to induce IFN-α synthesis. These findings support the idea that virus replication per se is not an absolute requirement for IFN induction and thus imply that membrane interactions between viral proteins and mononuclear cells provide sufficient stimuli for the induction process.

The coronavirus termed transmissible gastroenteritis virus (TGEV) is an enveloped RNA virus which induces acute and often fatal diarrhea in newborn pigs (7). We have shown that high levels of IFN-α are produced early after TGEV infection in the intestinal tract, lungs, serum, and urine, before the onset of clinical signs (10, 11). Several reports recently focused on in vitro IFN-α induction by TGEV and described its production after viral infection of porcine alveolar macrophages (14) or after exposure of blood lymphocytes to infectious virus (5), to UV-inactivated virus (15), or to TGEV-infected cells (2, 3). To analyze in more detail the interaction between lymphocytes and TGEV-infected cells resulting in IFN production, we have used an experimental system in which TGEV-infected, glutaraldehyde-fixed epithelial cells were incubated with lymphocytes to induce IFN. In the present report, we have shown that infected, fixed cells induce an early and strong IFN-α production in porcine as well as human or bovine lymphocytes. We have observed that two monoclonal antibodies (MAbs) directed against the minor viral glycoprotein E1 could block the IFN-α-inducing capacity of both TGEV-infected cells and the viral particle itself. This appears to be a newly recognized biological function of the coronavirus E1 protein.

MATERIALS AND METHODS

PBMC. Porcine, human, and bovine peripheral blood mononuclear cells (PBMC) were obtained from heparinized blood by Ficoll density centrifugation on Lymphoprep (density, 1.077; Nyegaard, Oslo, Norway) (3). Porcine blood samples were collected from 2- to 4-month-old animals lacking neutralizing anti-TGEV serum antibodies. PBMC were suspended at concentrations of 2.5×10^6 to 10×10^6/ml in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS).

Virus. As a source of TGEV, we used the cell-adapted Purdue 115 strain. Methods for propagation and titration have already been described (14).

Cell cultures. The epithelial pig kidney cell lines PD_5 and RPTG (14) were cultured in Dulbecco minimal essential medium (MEM) and MEM-Spinner supplemented with 10% FCS, respectively. The swine testis cell line ST was cultured in MEM plus 10% FCS. These cells were plated for 48 h in 24-well plates (Costar no. 3512) at a density of 5×10^5/ml (0.5 ml per well). TGEV infection was performed for 1 h in serum-free MEM at a multiplicity of infection of 1. Cultures were then incubated at 38°C overnight (unless otherwise stated) in medium supplemented with 5% normal calf serum.

Treatment of TGEV-infected cells with glutaraldehyde. TGEV-infected and control cells were treated with glutaraldehyde as described by Lebon et al. (19), as follows: monolayers were washed with MEM, incubated for 1 h at 4°C with 0.25% glutaraldehyde in phosphate-buffered saline, washed twice in phosphate-buffered saline, incubated for at least 1 h at 20°C with 3% glycine in phosphate-buffered saline, and washed with RPMI 1640 before addition of PBMC.

The characterization of murine MAbs directed against TGEV viral proteins was previously described (13). The anti-E1 MAbs, in particular, are of immunoglobulin G isotype and devoid of neutralizing activity.

A sheep anti-human IFN-α antiserum was kindly provided by C. Chany (Paris, France). It was shown to specifically neutralize porcine IFN-α (12). We used it at a final dilution of 1/100.

Induction of IFN. PBMC (5×10^6/ml unless otherwise stated) were cultured with TGEV-infected cell monolayers (4×10^6/ml) overnight (unless otherwise stated) at 37°C in RPMI plus 10% FCS. After cultivation, supernatants were harvested and assayed for IFN activity.

IFN assay. IFN was assayed on bovine MDBK cells using vesicular stomatitis virus as a challenge (10). A laboratory

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TABLE 1. Production of IFN by porcine PBMC cultured with TGEV-infected cells: comparison of nonfixed and glutaraldehyde-fixed monolayers

Inducer cells[a]	IFN titer[b] (U/ml) of PBMC added at time postinfection:		
	3 h	7 h	24 h
Fixed	<4	5,200	3,000
Nonfixed	5,200	5,200	8,100

[a] TGEV-infected RPTG cells. Fixed, Glutaraldehyde fixed.
[b] IFN titer after overnight incubation with PBMC (5×10^6/ml). IFN titers in culture fluids of PBMC incubated with uninfected cells were <4 U/ml.

standard porcine IFN was included in each assay. This standard was calibrated in MDBK cells with human international reference IFN B69/19 (National Institutes of Health, Bethesda, Md.). In our results, 1 U is equivalent to 1 IU of human IFN.

RESULTS

Induction of IFN by TGEV-infected cells. Porcine PBMC from seronegative animals were cultured with TGEV-infected monolayers of different porcine cell lines, and the supernatants were examined for antiviral activity. At the multiplicity of infection used, the time of appearance of a cytopathic effect was 10 to 15 h in the different cell lines tested. Monolayers were treated (or not) with glutaraldehyde at various times postinfection and then incubated overnight with PBMC. Table 1 shows the results of one representative experiment, in which a high level of IFN synthesis was detected in the culture fluids of infected cells incubated with PBMC. The IFN titers obtained when PBMC were cultured with fixed infected cells were only slightly lower than those obtained with nonfixed monolayers. A similar IFN-inducing effect was observed with the various TGEV-susceptible porcine cell lines tested: PD_5 and RPTG (and ST cells, not shown). Also, the IFN-inducing capacity of TGEV-infected cells was not restricted to porcine PMBC since human PMBC and, to a lesser degree, bovine PMBC were also induced to produce IFN (Table 2).

IFN produced by porcine PBMC after induction by glutaraldehyde-fixed infected epithelial cells was characterized as IFN-α because it was neutralized by anti-IFN-α antiserum (Table 3) and was acid stable (data not shown). In contrast, TGEV-infected nonfixed epithelial cells produced low amounts of nonneutralized IFN, presumably of the β type (Table 3).

Kinetics and dose effects of PBMC on IFN-α production after induction by glutaraldehyde-fixed cells. Time-course studies of IFN production showed that IFN was detected very early after the beginning of incubation with PBMC (averaging 1,000 U/ml at 3 h) and reached a maximum titer by around 10 h. The IFN yield slightly decreased after 26 h of incubation.

TABLE 2. Production of IFN by PBMC from different species cultured with glutaraldehyde-fixed, TGEV-infected PD_5 cells

PBMC concn (10^6/ml)	IFN titer[a] (U/ml) of PBMC species:		
	Porcine	Human	Bovine
2.5	330	1,000	110
5	1,700	1,000	330
10	5,200	5,200	330

[a] After overnight incubation.

TABLE 3. Characterization by anti-IFN-α antiserum of IFN induced by TGEV-infected RPTG cells

Glutaraldehyde-fixed inducer cells	PBMC added[a]	IFN titer (U/ml)	
		Without antiserum	With antiserum[b]
+	+	3,000	4
−	+	27,000	110
−	−	330	110

[a] Porcine PBMC (5×10^6/ml).
[b] IFN was titrated in the presence of 1/100-diluted sheep anti-IFN-α antiserum.

When infected monolayers were fixed with glutaraldehyde at various incubation periods after infection (1 to 18 h), the cells fixed before 6 h postinfection appeared to be poor IFN inducers whereas monolayers fixed between 8 and 18 h postinfection induced high IFN titers (Fig. 1).

Dose-response studies of various numbers of producer cells clearly indicated a positive correlation between PBMC concentrations, or ratios of PBMC per fixed cell, and IFN titers (Fig. 2).

Effects of MAbs specific to viral proteins on IFN induction. To identify the TGEV components involved in the IFN induction process, MAbs (13) directed against each of the three TGEV structural polypeptides were added to cocultures of porcine PBMC (5×10^6/ml) and TGEV-infected glutaraldehyde-fixed monolayers. Supernatant fluids were collected after overnight incubation and assessed for IFN. MAbs of different specificities were studied: (i) MAbs to the peplomer protein E2, and displaying neutralizing activity; (ii) nonneutralizing anti-E2 MAbs; (iii) nonneutralizing MAbs directed against nucleoprotein N; and (iv) MAbs directed against the transmembrane glycoprotein E1 and devoid of neutralizing activity (13). The IFN-α-inducing capacity of the monolayers was slightly, or not at all, modified by anti-E2 and anti-N MAbs and by two anti-E1 MAbs (9-34 and 3-60). However, a great reduction of IFN yield was observed with the anti-E1 MAbs 25-22 and 49-22 (Table 4) (e.g., from 1,700 to 4 U/ml).

Addition of MAbs 25-22 and 49-22 to PBMC infected with influenza virus did not abolish IFN induction (data not shown). These MAbs appeared therefore to be devoid of anti-IFN-α or anti-PBMC activity and, rather, to interact specifically with the IFN-α induction process. An obvious

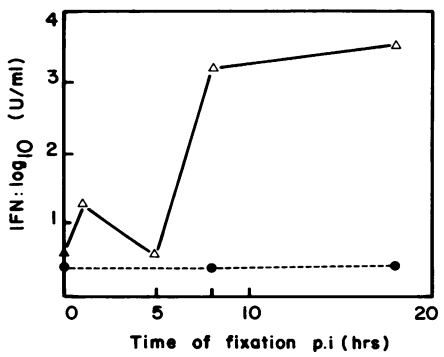

FIG. 1. IFN yield in PBMC: effect of time of cell fixation after infection. TGEV-infected PD_5 monolayers (△) or control monolayers (●) were incubated overnight with porcine PBMC (5×10^6/ml), and IFN was titrated in supernatant fluids as described in Materials and Methods.

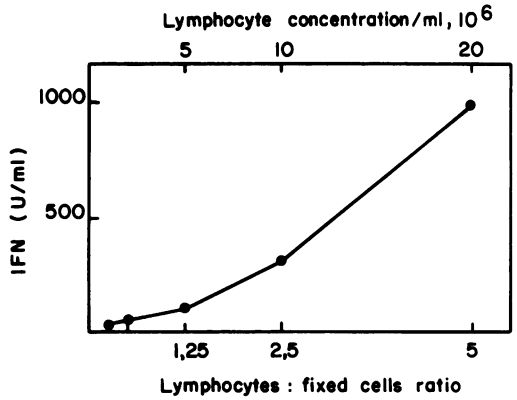

FIG. 2. Dose-response study of PBMC-fixed cells. Various PBMC concentrations were cultured overnight on replicates of glutaraldehyde-fixed, TGEV-infected PD_5 cells. IFN was titrated in supernatants.

dose-response correlation was observed between the amount of MAbs and IFN titers (Fig. 3a). A similar dose-response inhibition of IFN induction was observed on TGEV-infected living monolayers by MAbs 25-22 and 49-22 (Fig. 3b). Moreover, although devoid of neutralizing activity, these two MAbs could inhibit IFN-α induction by TGEV virus itself, either UV inactivated (data not shown) or fully infectious (98 to 99.9% inhibition) (Fig. 3c). These results indicate therefore that certain epitopes on the transmembrane glycoprotein E1 play a key role in IFN induction by TGEV and TGEV-infected cells.

DISCUSSION

It was previously shown that TGEV coronavirus induces IFN-α production in infected newborn pigs (10) as well as in cultures of pulmonary macrophages (14) or of lymphocytes (5) and, in this latter case, even after UV inactivation (15). The findings reported in this paper show that glutaraldehyde-fixed infected monolayers also induce high levels of IFN-α in naive porcine lymphocytes. The IFN-inducing ability is not restricted to the TGEV natural host, since we observed IFN production by human and bovine PBMC cultured with

TABLE 4. Effect of MAbs against TGEV structural proteins on IFN induction

MAb[a]	Specificity	Neutralizing activity	IFN yield[b] (% of control)
49-22	E1	−	2
25-22	E1	−	1.2
3-60	E1	−	100
9-34	E1	−	57
20-9	E2	+	100
51-13	E2	+	100
48-1	E2	+	100
25b21	E2	+	100
40-1	E2	+	33
5-2	E2	+	33
10-4	E2	+	33
11-20	E2	−	33
6-179	E2	−	100
78-17	E2	−	33
51-1	N	−	100
19-1	N	−	100
22-6	N	−	100

[a] MAbs were used as ascites fluids at a final dilution of 1/20. Detailed characteristics of MAbs have been described (13).

[b] PBMC (5×10^6/ml) were cultured overnight with fixed PD_5 monolayers, with or without MAb. Results are expressed as percent of IFN titer in the absence of MAb.

infected monolayers. The fact that potent IFN induction is achieved by fixed cells has already been described for other viruses such as HSV (1, 19) or dengue virus (9). These experiments strongly suggest that IFN-α induction is independent of virus penetration into producer cells (8, 17), but could rather be the consequence of interactions between membrane-associated viral antigens and lymphocyte membranes (1, 8, 17). It has been hypothesized that lymphocyte membrane-bound receptors, specific for IFN induction, can be triggered by viral antigens (17), although the precise mechanism of IFN induction by virus-infected cells remains to be elucidated.

Our results afford interesting information about the nature of the viral components involved in triggering IFN-α-producer cells. When 10 MAbs directed against each of the four major antigenic sites of viral structural protein E2 (6) and 3 MAbs directed against nucleoprotein N were tested, IFN yields were not significantly affected. In contrast,

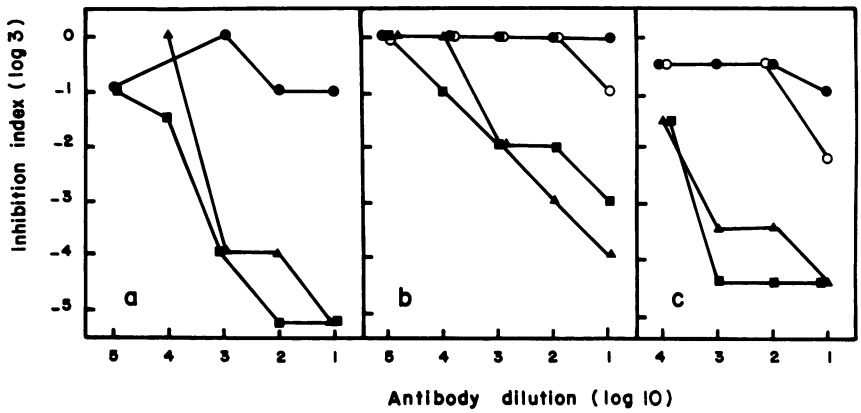

FIG. 3. Effect of anti-E1 MAbs on IFN induction by TGEV-infected cells. Monolayers were incubated overnight with porcine PBMC (5×10^6/ml) with various dilutions of MAb. Results were expressed as inhibition index: $\log_3$ (IFN titer with MAb) − $\log_3$ (IFN titer without antibody). MAbs: 49-22 (▲), 3-60 (●), 25-22 (■), 9-34 (○). PMBC were incubated with (a) glutaraldehyde-fixed monolayers, (b) nonfixed monolayers, or (c) infectious TGEV (0.2 PFU/cell). TGEV does not replicate in PBMC (15).

among four MAbs directed against E1, two of them could markedly decrease IFN-α induction. Since surface immunofluorescence control experiments indicated that both E2 (13) and E1 (data not shown) were expressed on the surface of infected cells, and because the anti-E2 MAbs used have high antibody titers (13), the negative results obtained with anti-E2 MAbs cannot be explained by their inability to react with TGEV-infected cells. We believe therefore that a portion of the TGEV transmembrane glycoprotein E1, in contrast to E2, plays a key role in IFN-α induction. Additionally, we have shown that the same two anti-E1 MAbs, although devoid of neutralizing activity, could block IFN-α induction by infectious or inactivated TGEV virions. In the case of HSV-infected cells, four MAbs directed against one each of three major surface glycoproteins were tested; only the two anti-gD MAbs could inhibit IFN induction, implying that HSV gD, also involved in the virus neutralization and cell-to-cell fusion processes, enabled infected cells to induce IFN-α in human lymphocytes (17).

According to its predicted amino acid sequence, the TGEV E1 glycoprotein is mainly buried in the lipid membrane (16). Thus, it is tempting to postulate that the interferogenic determinant should be located within the short (around 30 residues), protruding N-terminal domain of the protein. To confirm this hypothesis, we are selecting escape mutants by IFN-induction-blocking MAbs, with the hope of identifying the corresponding mutation(s) on the E1 gene. It will also be important to learn whether soluble E1 protein can trigger lymphocytes to produce IFN or if it must be associated in some way with a membrane.

Finally, the role of IFN-α produced by lymphocytes exposed to TGEV-infected cells in vivo remains to be investigated. Since IFN-α is synthesized early after contact between PBMC and infected cells, at low producer/inducer cell ratios (around 1), it is possible that IFN-α induced in vivo is released before the end of the first infectious cycle and could therefore rapidly protect uninfected cells. In addition, this IFN-α might activate neonatal natural killer cells (4) which in turn would help to limit the spread of virus infection (21). We believe that this alternative pathway for IFN-α induction, i.e., by direct interactions of viral proteins with circulating lymphocytes, might be of importance in most viral infections and deserves more attention.

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