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Replication of Porcine Parvovirus in Peripheral Blood Lymphocytes, Monocytes, and Peritoneal Macrophages

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Porcine peripheral blood lymphocytes (PBL), peripheral blood monocytes, and peritoneal macrophages were examined for their ability to support porcine parvovirus (PPV) replication. The cell cultures were infected with the NADL-2 strain of PPV at 0.1 multiplicity of infection. PBL cultures were stimulated with the following phytomitogens: phytohemagglutinin M, concanavalin A, and pokeweed mitogen. Unstimulated PBL cultures infected with PPV and uninfected PBL stimulated with phytomitogens served as controls. All cultures were examined daily for PPV-specific immunofluorescence and hemagglutinin. PPV replicated in PBL cultures stimulated with all phytomitogens. Both viral hemagglutinin in culture fluids and nuclear immunofluorescence in cells were detected. In contrast, unstimulated PBL did not support viral replication; however, PPV antigen was detected in the cytoplasm. PPV persisted in unstimulated PBL for 21 days (duration of the experiment) without replication, but replicated each time with the addition of phytohemagglutinin M at 0, 3, 7, 14, and 21 days after infection. Uninfected PBL stimulated with phytomitogens lacked both viral hemagglutinin and immunofluorescence. Simultaneous detection of lymphocyte surface marker and viral antigens in pokeweed mitogen-stimulated PBL revealed that both T and non-T cells (B and null cells) are able to support PPV replication. Peripheral blood monocytes and peritoneal macrophages phagocytized PPV but did not support virus replication.

Porcine parvovirus (PPV), a nondefective parvovirus, is a major cause of reproductive failure in swine (11-13). The multiplication of PPV is apparently dependent on the cellular deoxyribonucleic acid synthesis phase and thus requires actively dividing cells for maximum viral replication (4, 11). PPV has been isolated from buffy-coat leukocytes (10), and PPV antigens have been detected in lymphoid tissues (2, 13). However, the replication of PPV in leukocyte cultures or the characterization of leukocyte type or types supporting virus replication has not been reported.

The purpose of the present report is to describe our studies on PPV replication in peripheral blood lymphocytes (PBL), peripheral blood monocytes (PBM), and peritoneal macrophage (PM) cultures.

MATERIALS AND METHODS

Virus. Strain NADL-2, a cell culture-adapted strain of PPV, was propagated in porcine fetal kidney (PFK) cell cultures (10). Stock virus consisted of infectious culture fluid at cell culture passage 6 and had a titer of $10^{6.5}$ 50% cell culture infective doses per 0.2 ml.

Cell cultures. PFK cell cultures were propagated in Eagle minimal essential medium supplemented with nonessential amino acids, 0.25% lactalbumin hydrolysate, 1 mM sodium pyruvate, 20% bovine fetal serum, 100 U of penicillin per ml, and 100 μg of streptomycin per ml. Lymphocytes, monocytes, and macrophages were cultured in RPMI 1640 medium containing 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer, 20% bovine fetal serum, and antibiotics.

PBM. Mononuclear cells were separated from heparinized peripheral blood on Ficoll-Hypaque gradients by the method of Boyum (1), washed twice with Hanks balanced salt solution, and suspended at a cell concentration of 10^6 in RPMI 1640 culture medium supplemented with 20% bovine fetal serum and antibiotics. Monocytes were depleted by adherence to plastic dishes at 37°C for 1 h (18). Nonadherent cells were comprised of >95% lymphocytes. To obtain PBM cultures, 2×10^6 mononuclear cells were dispensed in Leighton tubes with cover slips. After incubation at 37°C for 1 h, nonadherent cells were discarded, and adherent cells were cultured in RPMI 1640 medium supplemented with 20% bovine fetal serum and antibiotics.

PM. A 20-ml amount of 10% proteose peptone (Difco Laboratories, Detroit, Mich.) in water was injected intraperitoneally into an 8-week-old pig free of hemagglutination (HA)-inhibiting antibodies to PPV.

After 3 days, PM were collected by lavage of the peritoneal cavity with 200 ml of RPMI 1640 culture medium containing 50 IU of heparin per ml. The cells were washed once with medium supplemented with 20% bovine fetal serum and antibiotics. A 2-ml amount of cell suspension was pipetted into Leighton tubes with cover slips. After 1 h of incubation at 37°C, nonadherent cells were discarded, the monolayers were washed twice with Hanks balanced salt solution, and fresh medium was added.

Identification of mononuclear cells. Monocytes and macrophages were identified by latex phagocytosis (15). Cells ingesting three or more latex beads were identified as monocytes or macrophages. T lymphocytes were identified by a sheep erythrocyte rosette assay as described for cattle (15). Approximately 60 to 70% of porcine PBL formed rosettes with this technique.

Phytomitogens. Phytohemagglutinin M (PHA; Difco Laboratories) was used at a concentration of 5 μl/ml of culture. Pokeweed mitogen (PWM; GIBCO Laboratories, Grand Island, N.Y.) and concanavalin A (ConA; Calbiochem, LaJolla, Calif.) were added at a concentration of 10 μg/ml of culture.

Assay of virus and viral antigens. Samples were examined for infectious virus by inoculating PFK cultures and subsequent examination by immunofluorescence (10). To examine PBL for immunofluorescent antigen, cell smears were prepared, air dried, fixed in acetone, and stained with fluorescein-conjugated antiserum to PPV. The percentage of positive cells was determined by counting a minimum of 500 cells. Viral hemagglutinin in culture fluids was detected by HA with guinea pig erythrocytes (10).

Infection of cells. PBL cultures were infected with the NADL-2 strain of PPV at 0.1 multiplicity of infection. To infect PBM and PM cultures, we added 0.2 ml of stock PPV directly into the medium. After 4 h of incubation at 37°C, the cells were washed twice with Hanks balanced salt solution to remove the unadsorbed virus, and fresh medium was added. Phytomitogens were added to PBL cultures at various time intervals after infection. Uninfected and infected cultures were periodically examined for extracellular HA activity, infectious virus, and PPV-associated immunofluorescence.

RESULTS

Replication of PPV in lymphocytes. The following experiments were designed to examine the ability of PBL to support PPV replication. In experiment 1, PBL were infected with PPV and either left unstimulated or stimulated with phytomitogens immediately after infection. Uninfected PBL cultures stimulated with phytomitogens served as controls. As shown in Fig. 1, unstimulated PBL did not support viral replication. PPV antigen was detected by immunofluorescence in the cytoplasm of unstimulated PBL immediately after infection. However, neither HA activity in the culture fluids nor nuclear immunofluorescence in the cells was detected. In contrast, PPV replicated in PBL cultures

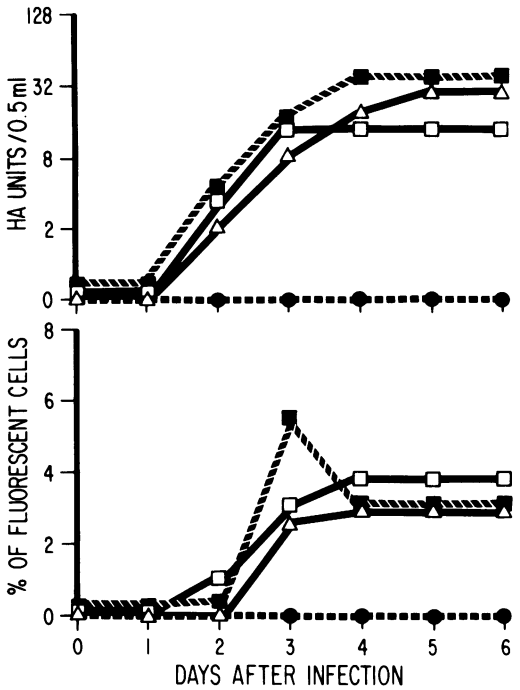

FIG. 1. *Replication of PPV in porcine PBL. Cells were infected with PPV, cultured with PHA (■), ConA (□), or PWM (▲) or without any phytomitogen (●), and examined daily for extracellular viral hemagglutinin and PPV-specific nuclear immunofluorescence.*

stimulated with PHA, PWM, and ConA. Both viral hemagglutinin and nuclear immunofluorescence (Fig. 2) were observed as early as 2 days after infection. Peak viral hemagglutinin titers (16 to 32 HA units per 0.5 ml) were reached at 3 to 5 days. Although all mitogens stimulated virus replication, the highest percentage (5.5%) of cells producing virus was detected in PHA-stimulated cultures 3 days after infection. The lack of HA and immunofluorescence in uninfected PBL cultures stimulated with phytomitogens shows the specificity of the HA activity and immunofluorescence. Additionally, immunofluorescence in PPV-infected cultures was blocked by PPV-immune serum but not by nonimmune serum.

In experiment 2, PBL were stimulated with phytomitogens for 2 days before infection. They were then infected with PPV and recultured for an additional 6 days with the respective phytomitogens. As shown in Fig. 3, PPV replicated in phytomitogen-stimulated PBL cultures but not in unstimulated cultures. Viral hemagglutinin was detected as early as 1 day after infection in culture fluids of PHA- and ConA-stimulated PBL cultures and 2 days after infection in those

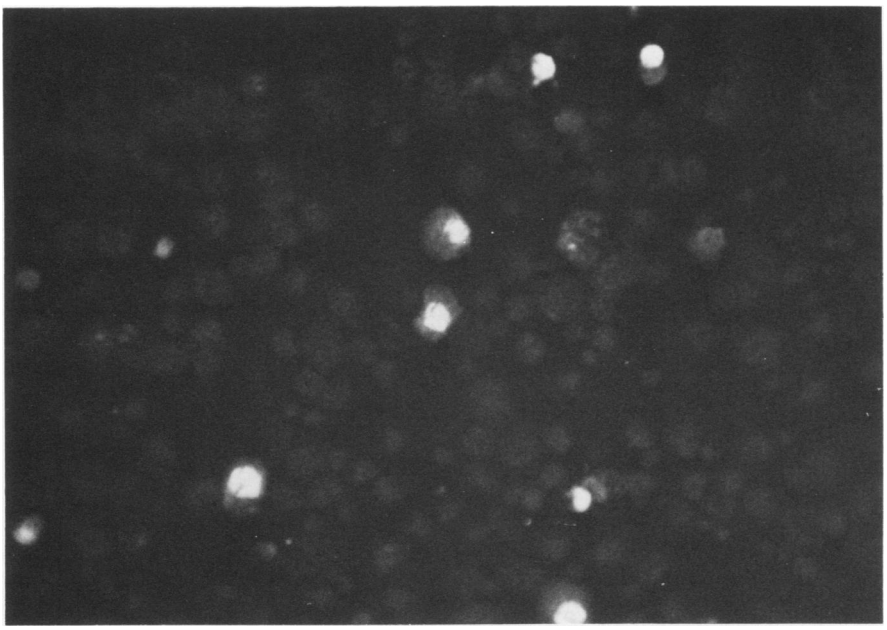

FIG. 2. *Porcine PBL infected with PPV, stimulated with PHA, and stained with porcine anti-PPV conjugate 3 days after infection. Infected cells with nuclear fluorescence are evident against the background of uninfected cells.*

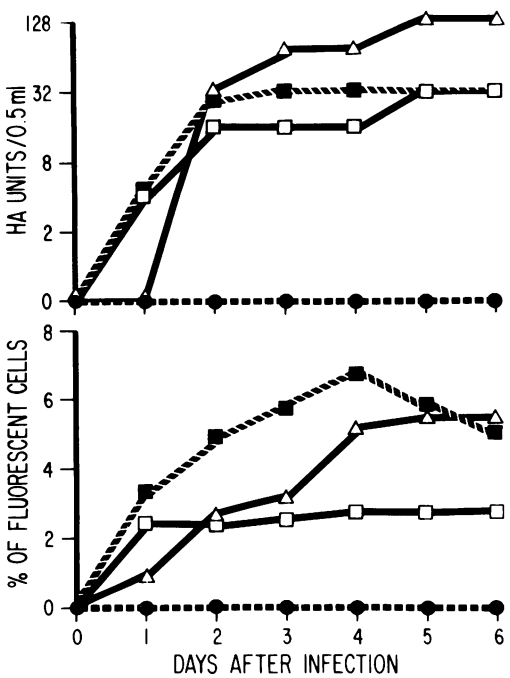

FIG. 3. *Replication of PPV in porcine PBL. Cells were cultured with PHA (■), ConA (□), or PWM (▲) or without any phytomitogen (●) 2 days before infection with PPV, recultured with respective phytomitogens after infection, and examined daily for extracellular viral hemagglutinin and PPV-specific nuclear immunofluorescence.*

of PWM-stimulated cultures. Virus-producing cells with nuclear fluorescence were evident in cultures stimulated with all mitogens 1 day after infection. Again, PHA induced the highest percentage (7%) of virus-producing cells.

In the previous experiments, PPV did not replicate in unstimulated PBL cultures; however, viral antigen was detected in their cytoplasm by immunofluorescence. To determine whether virus persisted in these cultures and whether it could replicate after mitogenic stimulation, PBL were infected with PPV and cultured for 21 days. PHA was added to a part of cultures at 0, 3, 7, 14, and 21 days after infection, and cultures were examined for viral antigens 3 days after mitogenic stimulation. PPV did not replicate in unstimulated PBL cultures; however, the virus replicated each time after addition of PHA. Titers of 16 to 32 HA units per 0.5 ml in culture fluids and nuclear fluorescence in 3 to 5% of cells were detected.

Characterization of lymphocyte subpopulation supporting virus replication. To determine the virus-producing lymphocyte subpopulation, PBL were cultured with PWM, a T- and B-cell mitogen (5), for 3 days and simultaneously examined for T cells by E-rosette assay and for PPV by immunofluorescence. Both rosette-forming (3.5%) and non-rosette-forming (3%) cells contained nuclear fluorescence; thus, both T and B cells are able to support viral replication.

Replication of PPV in PBM and PM. Neither PBM nor PM cultures supported viral replication. Neither HA activity in the culture fluids nor immunofluorescence was observed. PPV-specific immunofluorescence was observed, however, in the cytoplasm of monocytes (Fig. 4) and macrophages immediately after infection. PPV antigen persisted in these cultures for 21 days (duration of the experiment) without replication; at that time, infectious virus was reisolated.

DISCUSSION

Results of the present study show that PPV can replicate in PBL but not in PBM or PM. PPV was able to infect unstimulated PBL; however, virus replicated only after mitogenic stimulation. The enhancing effect of mitogens on virus replication was not surprising when we consider the nature of the infecting virus. Nondefective parvoviruses are highly dependent on cell division for replication (9, 17). Phytomitogens induce blast transformation and increase deoxyribonucleic acid synthetic activity of lymphocytes, providing a permissive state for PPV replication. A similar enhancing effect of phytomitogens on the replication of adenovirus (8), herpesvirus (7), measles virus (6), polio virus (19), and vesicular stomatitis virus (3) in lymphocytes has also been reported.

In the present study, we determined that PPV can persist in unstimulated PBL for a long time; the virus replicates only after mitogenic stimulation. The possible significance of this finding is that PPV could persist in lymphoid cells in pigs for long periods, after which virus could replicate in lymphocytes proliferating in response to antigenic stimuli. Supporting evidence for the role of lymphocytes in PPV persistence in pigs is that PPV can be isolated from buffy-coat leukocytes and lymphoid tissues despite high levels of circulating antibodies (P. S. Paul, unpublished observations). Additionally, virus-infected lymphocytes may also play a key role in virus dissemination throughout the host, including the central nervous system. Evidence for such a pathway has been shown in encephalomyelitis induced by the distemper virus in dogs (16). The mechanism of transplacental transmission of PPV in pigs is unknown (14), but it appears unlikely that lymphocytes play a major role in fetal infection. However, transfer of PPV-infected lymphocytes from mother to fetus under certain conditions cannot be excluded.

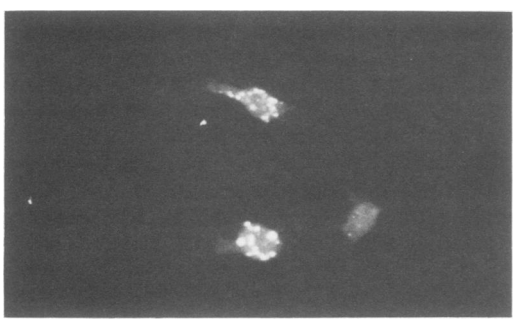

FIG. 4. *Cytoplasmic fluorescence in PBM infected with PPV, cultured, and stained with porcine anti-PPV conjugate 3 days after infection.*

We found that PPV replicates in diverse types of lymphocyte populations. In the mitogenic stimulation experiments, virus replication was stimulated by T-cell mitogens PHA and ConA, as well as by the T- and B-cell mitogen PWM (5). Mitogenic stimulation data, along with the demonstration of viral antigen in T cells and non-T cells, comprised of B and null cells, indicate that both T and B lymphocytes can support PPV replication. Our observations agree with those in in vivo studies by Cutlip and Mengeling (2) in which PPV antigen was detected in the thymus and in the germinal centers of lymph nodes, representing T- and B-lymphocyte-populated areas, respectively. Conclusive evidence for PPV replication in T and B lymphocytes, however, awaits infection of highly purified lymphocyte populations.

On the basis of our in vitro studies, macrophages may play an important role in PPV pathogenesis in that they phagocytize PPV and disseminate the virus to susceptible cells without supporting viral replication. The in vivo detection of PPV antigen in the cytoplasm of a macrophage-like cell by Mengeling and Cutlip (13) supports this hypothesis. We do not know whether activated macrophages can support PPV replication. Our studies show that lymphocytes undergoing blastogenic transformation are required for PPV replication and that lymphocytes and macrophages may play a role in PPV persistence in pigs.

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LITERATURE CITED

1. **Boyum, A.** 1968. Isolation of mononuclear cells and granulocytes from human blood. Isolation of mononuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation. Scand. J. Clin. Lab. Invest. Suppl. 97 **21**:77-89.
2. **Cutlip, R. C., and W. L. Mengeling.** 1975. Experimentally induced infection of neonatal swine with porcine parvovirus. Am. J. Vet. Res. **36**:1179-1182.
3. **Edelman, R., and E. E. Wheelock.** 1966. Vesicular stomatitis virus replication in human leukocyte cultures:

enhancement by phytohemagglutinin. Science **154:** 1053-1055.

4. **Hallauer, C., G. Siegl, and G. Kronauer.** 1972. Parvoviruses as contaminants of permanent human cell lines. III. Biological properties of the isolated viruses. Arch. Gesamte Virusforsch. **38:**366-382.
5. **Janossy, G., and M. F. Greaves.** 1971. Lymphocyte activation. I. Response of T and B lymphocytes to phytomitogens. Clin. Exp. Immunol. **9:**483-498.
6. **Joseph, B. S., P. W. Lampert, and M. B. A. Oldstone.** 1975. Replication and persistence of measles virus in defined subpopulations of human leukocytes. J. Virol. **16:**1638-1649.
7. **Kleinman, L. F., S. Kibrick, F. Ennis, and P. Polgar.** 1972. Herpes simplex virus replication in human lymphocyte cultures stimulated with phytomitogens and anti-lymphocyte globulin. Proc. Soc. Exp. Biol. Med. **141:**1095-1099.
8. **Lambriex, M., and J. van der Veen.** 1976. Comparison of replication of adenovirus type 2 and type 4 in human lymphocyte cultures. Infect. Immun. **14:**618-622.
9. **Margolis, G., and L. Kilham.** 1965. Rat virus, an agent with an affinity for the dividing cells, p. 361-367. *In* D. C. Gajdusek, C. J. Gibbs, Jr., and M. Alpers (ed.), Slow, latent, and temperate virus infections. National Institute of Neurological Diseases blindness monograph no. 2. National Institute of Neurological Diseases, Bethesda, Md.
10. **Mengeling, W. L.** 1972. Porcine parvovirus: properties and prevalence of a strain isolated in the United States. Am. J. Vet. Res. **33:**2239-2248.
11. **Mengeling, W. L.** 1975. Porcine parvovirus, p. 432-446. *In* H. W. Dunne and A. D. Leman (ed.), Diseases of swine. The Iowa State University Press, Ames.
12. **Mengeling, W. L.** 1978. Prevalence of porcine parvovirus-induced reproductive failure: an abattoir study. J. Am. Vet. Med. Assoc. **172:**1291-1294.
13. **Mengeling, W. L., and R. C. Cutlip.** 1976. Reproductive disease experimentally induced by exposing pregnant gilts to porcine parvovirus. Am. J. Vet. Res. **37:**1393-1400.
14. **Mengeling, W. L., R. C. Cutlip, and D. Barnett.** 1978. Porcine parvovirus: pathogenesis, prevalence and prophylaxis, p. KA14. *In* M. Herak and M. Sviben (ed.), Proceedings of the Fifth World International Pig Veterinarian Society Congress. Zrinski-Čakovec.
15. **Paul, P. S., D. R. Sengles, C. C. Muscoplat, and D. W. Johnson.** 1979. Enumeration of T cells, B cells and monocytes in peripheral blood of normal and lymphocytotic cattle. Clin. Exp. Immunol. **35:**306-316.
16. **Summers, B. A., H. A. Greisen, and M. J. G. Appel.** 1978. Possible initiation of viral encephalomyelitis in dogs by migrating lymphocytes infected with distemper virus. Lancet **ii:**187-189.
17. **Tattersall, P.** 1972. Replication of the parvovirus MVM. I. Dependence of virus multiplication and plaque formation on cell growth. J. Virol. **10:**586-590.
18. **Territo, M. C., D. W. Golder, and M. J. Cline.** 1976. Macrophage activation and function, p. 142-147. *In* N. R. Rose and H. Friedman (ed.), Manual of clinical immunology. American Society for Microbiology, Washington, D.C.
19. **Willems, F. T. C., J. L. Melnick, and W. E. Rawls.** 1969. Replication of poliovirus in phytohemagglutinin-stimulated human lymphocytes. J. Virol. **3:**451-457.