NOTE Virology

Suppression of the Proliferation of Mouse Splenocytes by Pseudorabies Virus

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(Received 14 April 2000/Accepted 7 August 2000)

ABSTRACT. Pseudorabies virus (PRV) infection in resistant swine caused immunosuppression which sometimes resulted in secondary infection by other viruses or bacteria. However the mechanism of the immunosuppression is not well understood. In this study, the effect of PRV on the immune system was examined in the mouse model. Splenocytes or lymphocytes prepared from the spleen of BALB/c mice were incubated in vitro with mitogen, and the ability of cells to proliferation was measured. When the cells were incubated with PRV, the ability of cells to proliferate was inhibited, although PRV did not multiply in the lymphocytes. UV-inactivated PRV also suppressed the proliferation of mice splenocyte. This result suggests that the structural component of PRV virion might cause the immunosuppression.

KEY WORDS: immunosuppression, mouse, pseudorabies virus.


Pseudorabies virus (PRV), an etiological agent of Aujeszky's disease of swine, belongs to herpesviridae, alphaherpesviruses subfamily. It has been suggested that some of the alphaherpesviruses are immunosuppressive [2, 9]. It has been known that higher incidents of pneumonia caused by bacteria or mycoplasma were observed in swine herds infected with PRV [4]. Swine experimentally co-infected with PRV and several bacteria or mycoplasma suffered more severe case of pneumonia [6, 17, 18], suggesting that PRV is also immunosuppressive. However, the mechanism of the immunosuppression caused by PRV is not well understood.

In this study, we examined the effect of PRV on the immune system in the mouse model. The mouse model has been used to study immunity against PRV infection [1] and to assess the protective effect of DNA vaccine against PRV challenge [7, 8]. We found that PRV infection of mouse splenocytes in vitro resulted in a reduction of the mitogenic activation by concanavalin A (Con A) and lymphopolysaccharide (LPS) although there was no sign of PRV growth in mouse splenocytes. We also found that UV-inactivated PRV had a similar inhibitory effect on mouse splenocytes.

Porcine kidney cells (CPK) [13], grown in Eagle's MEM (autoclavable, Nissui) containing 7.5% of fetal bovine serum (FBS) and 60 μg/ml kanamycin, were infected with pseudorabies virus (PRV), Indiana strain and incubated at 37°C for 48 hr. The supernatant of the infected cell culture was harvested, centrifuged to remove cell debris, aliquoted and stored at −80°C as a virus stock. Mouse splenocytes were prepared as follows. Spleens obtained from 8–10 weeks old female BALB/c mice were homogenized in phosphate buffered saline (PBS). The erythrocytes in the harvested cell suspension were lysed with 0.83% NH₄Cl in 0.85 M Tris buffer (pH 7.65). After being washed three times with PBS, the splenocytes were re-suspended with RPMI 1640 (Gibco BRL) supplemented with 10% FBS, 14 mM HEPES and 5 × 10⁻⁵ M 2-mercaptoethanol.

Splenocytes were suspended at 2 × 10⁵ cells/ml with the supplemented RPMI 1640 containing 10 μg/ml of Con A or LPS, and distributed into 96-well plate at 100 μl/well (2 × 10⁴ cells/well). And then 100 μl/well of supplemented RPMI1640 with or without 2 × 10¹ pfu of PRV (PRV infected samples and mock-infection approximately). So the multiplicity of infection (moi) of PRV infected samples resulted in 1.0. After incubated at 37°C for 42 hr, the splenocytes were labeled with 1 μCi/well of ³H-thymidine for 6 hr. Cells were harvested onto glass filter papers and the incorporation of ³H-thymidine into cells was measured in a scintillation counter. Con A is one of the T cell mitogens and LPS is known to induce cytokine release of macrophage. In this assay system, therefore, lymphocytes in the splenocytes preparation is directly activated by Con A, while LPS activates macrophages (monocytes) and cytokines released by activated macrophage induce proliferation of lymphocytes. As shown in Fig. 1A and B, PRV infection suppressed the proliferation of splenocytes stimulated by both Con A and LPS. It was confirmed that Eagle's MEM and supernatant of CPK cell culture without PRV infection did not suppress the proliferation of these cells (data not shown). In another experiments, splenocytes were activated with Con A or LPS for 42 hr and then infected with PRV but no inhibitory effects of PRV was observed in this condition (data not shown).

It has been reported earlier that PRV infection has suppressive effect on the function of alveolar macrophages of porcine [5, 11, 16]. The splenocytes preparation we used in the experiment in Fig. 1A and B contained lymphocytes and macrophages (monocytes). To determine whether the suppressive effect of PRV on mouse splenocytes was due to the direct effect on lymphocytes or is caused via the effect on monocytes in the splenocytes preparation, we designed following experiments. The splenocytes harvested as described...
the lymphocytes were resuspended with 1 ml of supplemented RPMI1640 containing 10 µg/ml of ConA, and 3H-thymidine incorporation into lymphocytes was measured as described above. As shown in Fig. 1 C, PRV reduced the incorporation of 3H-thymidine in Con A stimulated lymphocytes. Lymphocytes without Con A stimulation incorporated 3H-thymidine at low level but PRV infection did not affect this low level thymidine incorporation (data not shown). These results suggest that PRV infection to lymphocytes caused the inhibition of lymphocyte activation by the mitogen and this inhibition is not the secondary effect of PRV infection to monocytes.

In the experiment in Fig. 2A, PRV was inactivated by exposing to UV right (GL-30, National) so that no plaque forming virus survived. Using the inactivated PRV, the effect of UV inactivated PRV on the proliferation of mouse splenocytes was investigated as same as the experiment indicated by Fig. 1C. Although the effect was smaller than that of live PRV, it is clearly seen that UV exposed PRV also reduces the incorporation of 3H-thymidine into Con A activated splenocytes. The fact that UV inactivation of PRV had the inhibitory effect suggest that the inhibition is not due to only infectious PRV but also to one of the viral components present in the viral stock. In the next experiment, PRV stock was centrifuged at 100,000 g for 2 hr on 80% (w/v) sucrose cushion and the pellet which contained virions was re-suspended with fresh media, aliquoted and stored at −80°C. The supernatant was filtered through a 0.45 µm filter (MILLIPORE, U.S.A.), aliquoted and stored at −80°C. The supernatant fraction contained large quantities of glycoproteins, which were not integrated into virions, fragmented envelope and defective virions. The lymphocytes preparation without monocytes were infected with the virion fraction or the supernatant fraction and washed as same as the experiment indicated by Fig. 1C. And then the thymidine incorporation was measured as described above. As shown in Fig. 2B, the purified virion fraction reduced the incorporation of 3H-thymidine into lymphocytes but the supernatant fraction was not inhibitory at all. These results suggested that the presence of virion is necessary to inhibit the proliferation of lymphocytes.

Next, we examined whether PRV replicates in mouse lymphocytes. The enriched lymphocytes were resuspended at 1 × 10^6/ml with RPMI 1640. Monocytes containing in splenocytes suspension were allowed to adhered to tissue culture dishes by incubating in a tissue culture dish for 2 hr and non-adhering lymphocytes containing splenocytes suspension were harvested. After repeating this procedure for three times, the lymphocytes preparation contained less than 1% of monocytes when the cell morphology was assessed under a microscope after Giemsa's staining. And then 1 × 10^6 of harvested lymphocytes were suspended with the supplemented RPMI 1640 with or without 1 × 10^5 pfu of PRV (PRV infected samples and mock-infection, approximately). So the moi of infected samples resulted in 1.0. After incubated 37°C for 3 hr, the lymphocytes were washed with PBS to remove viruses in the medium and resuspended with 1 ml. And then
Fig. 2. Effect of UV inactivated PRV and purified virions on proliferation of mouse lymphocytes. A) After incubated with UV inactivated PRV for 3 hr at appropriate moi (moi before inactivation), the lymphocytes were washed and incubated in medium containing 10 μg/ml of Con A. After incubated for 48 hr, the incorporation of [methyle-3H]-thymidine was measured. The incorporation of [methyle-3H]-thymidine into cells were standardized as % of mock infected control. Vertical bars indicate the standard error of means. B) After incubated with purified PRV suspended with 1 ml of supplemented RPMI1640 or 1 ml of supernatant fractions for 3 hr at the concentration of 10⁶ cells/ml, the lymphocytes were washed and incubated in medium containing 10 μg/ml of Con A. Thymidine incorporation was measured as above.

Fig. 3. The replication of PRV on mouse lymphocytes. Lymphocytes were suspended at 1 x 10⁶/ml in the supplemented RPMI1640 with or without 10 μg/ml of Con A and seeded into 3.5 cm dish at 1 ml/dish. Each culture was infected with PRV at moi of 1. After incubated for appropriate hours, the virus titer was evaluated by plaque titration on CPK cells. As the negative control, virus titer incubated in the medium without any cells were also measured. ○: Virus titer in lymphocytes suspension with Con A. ●: Virus titer in lymphocytes suspension without Con A. □: Virus titer in medium with Con A and without any cells. ▼: Virus titer in medium without Con A and without any cells.
Kit” (Boehringer Mannheim). Neither necrosis nor apoptosis was increased in PRV infected mouse lymphocytes when compared with mock-infected control (data not shown).

It has been reported that PRV suppressed the proliferation of lymphocytes and multiples in the swine lymphocytes [3]. Our present study demonstrated that PRV suppresses the proliferation of mouse lymphocytes in the absence of viral penetration or multiplication. It may be possible that attachment of PRV to lymphocytes is enough to suppress the proliferation of lymphocytes. Bovine herpesvirus type 1 (BHV-1) causes immunosuppression and it has been reported that the attachment of BHV-1 to bovine peripheral blood mononuclear cells is enough to induce the apoptosis in these cells [10]. However, we did not observe the increase of apoptosis in PRV infected mouse lymphocytes. We also failed to detect any suppressive effect of BHV-1 on the proliferation of mouse lymphocytes (data not shown). These results may suggest that the mechanism of immuno-suppression by PRV may be different from that by BHV-1.

REFERENCES