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Pseudorabies Virus HLJ Strain Suppresses Peripheral Blood T and B Lymphocytes in Mice

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ABSTRACT

Background: Pseudorabies virus (PRV), responsible for pseudorabies, is one of the most important pathogen in swine industry. PRV infection suppresses interferon-β (IFN-β) and tumor necrosis factor-α (TNF-α) expression, which consequently results in an immune escape in the host.

Methods: In this study, distribution of peripheral blood T and B cell sub-populations was analyzed by flow cytometry after infecting mice with 1×10^5 $TCID_{50}$ PRV.

Result: We found that the levels of $CD3^+$ T, $CD4^+$ T and $CD8^+$ T lymphocyte in infection group were significantly decreased at 24 hpi ($p<0.01$) and remained at a low level until 72 hpi compared to the control group. Interestingly, the levels of $CD19^+$ T lymphocyte were significantly lower after 24 hpi ($p<0.01$) and lasted until 48 hpi, then increased sharply and then dropped at 96 hpi. Collectively, these results indicated that PRV infection could play a negative role in adaptive immunity in mice by decreasing the levels of B and T lymphocytes.

Key words: B lymphocyte, Flow cytometry, Mice, Pseudorabies virus, T lymphocyte.

INTRODUCTION

Pseudorabies, also called Aujeszky's Disease (AD), is an acute, highly contagious and immunosuppressive swine disease caused by pseudorabies virus (PRV), which is characterized by nervous and respiratory symptoms. PRV belongs to the genus *Varicellovirus* in the family of *Herpesviridae* and its genome consists of a linear double-stranded DNA (Papageorgiou *et al.*, 2017). PRV infect a wide variety of domestic animals, including swine, cattle, sheep, goats, cats, dogs and even in wildlife (Minamiguchi *et al.*, 2019). It was recently reported that, the human acute encephalitis was caused by a novel PRV variant strain (Hou *et al.*, 2022). However, swine is the only natural host of this virus because it's the only animal species that survives after an acute primary infection, therefore, possing a treat to other livestock and crop farmers (Papageorgiou *et al.*, 2017). In addition, PRV can also infect rodents, including mice and rats (Müller *et al.*, 2011). Therefore, as a susceptible animal, mice have been used as models to investigate PRV pathogenesis.

Lymphocytes, which play an important role in regulating the immune function of the body, are divided into T lymphocytes ($CD3^+$) mainly involved in cellular immunity and B lymphocytes ($CD19^+$) mainly involved in humoral immunity. T lymphocytes are divided into helper T lymphocytes ($CD3^+CD4^+$) and suppressive/cytotoxic T lymphocytes ($CD3^+CD8^+$). The latter two types of lymphocytes form a T cell network by inducing and restricting each other, which critically function in regulating cellular immune response and maintain immune balance. $CD4^+CD8^+$ double positive T cells refer to the simultaneous expression of CD4 immature T cells with CD8 antigen. They are widely distributed in many peripheral blood and immune organs of animals. They can be affected by mitogen, pathogen and toxic substances stimulation and proliferation.

Previous studies have suggested that PRV infection has an immunosuppressive effect on the host at the leukocyte level (Deng *et al.*, 2022). However, whether PRV infection affects the cellular and humoral immunity remains unclear. Therefore, in this study, we aimed to detect the effect of PRV infection on peripheral blood T/B lymphocytes in mice through flow cytometry analysis.

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MATERIALS AND METHODS

Cells, viruses and antibodies

PK 15 (Porcine kidney) cells were maintained in Dulbecco's modified Eagle Medium (DMEM) containing 10% FBS (fetal bovine serum). PK 15 and DMEM were obtained from the laboratory of Tongren Polytechnic College. These cells were

incubated in 37°C in a humidified 5% CO_2 incubator. The PRV HLJ strain (Genbank accession number: MK080279.1) was kindly provided by Prof. Jingfei-Wang from Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences. Antibodies against mouse CD3E PE-CY7 (552774), mouse CD4 APC, mouse CD8a FITC and FITC Rat Anti-Mouse CD19 (557398) antibodies were purchased from BD Pharmingen company.

Viral infection and titration detection

PK15 cells were infected with PRV at a multiplicity of infection (MOI) of 0.5. After incubation at 37°C for 1 h, the cells were washed three times with phosphate buffered saline to remove the unattached virus and then cultured in DMEM supplemented with 2% FBS at 37°C. PBS was purchased from Beijing Solarbio Science and Technology Company. Tissue culture infectious dose 50 ($TCID_{50}$) was used to detect viral titers according to the Reed and Muench formula (Reed and Muench, 1938).

Animal experiment

84 eight-week-old female BALB/c mice were bought from Experimental Animal Corporation of Dossy Biological Technology Company (Chengdu, Sichuan, China). After one week, the mice were divided randomly into control group (n=10) and PRV infected group (n=50) at 36 h, 48 h, 72 h and 96 h post-infection. Animals were kept at a room temperature of 22±3°C and a relative humidity of 50±10% with centralised ventilation conditioning through the HVAC system(Srinivasan *et al.*, 2021). The mice in all groups were fed with a nutritionally balanced diet. Water was provided ad libitum. Mice was infected with the PRV-HLJ strain by subcutaneous injection at a dose of 1×10^5 $TCID_{50}$ according to the method as previously described (Zang *et al.*, 2015).

To determine the mortality rates of PRV-HLJ strain inoculated mice, another 24 eight-week-old female BALB/c mice were randomly divided into two groups and raised in separate isolator. The mice in the second group (n=6) were injected with 0.1 mL PBS as a negative control. The remaining mice served as the infection group and the PRV injection dose was 100 µl 1×10^5 TCID50 . Signs of disease and death in all groups were observed within a week.

Determination the change of T/B lymphocytes by flow cytometry

The changes of peripheral blood T/B lymphocytes were detected by using a Z15 flow cytometry in Yangkester Co., Ltd. 100 µL anticoagulant was added in a flow tube and 1µL of CD3, CD4 APC and CD8 antibody was added respectively and then stained in dark room at 4°C for 30 min. Another 100 µL anticoagulant is added in a new flow tube and 1 µL FITC labelled rat anti mouse CD 19 was added and stained in dark at 4°C for 30 min. Hemolysin working solution was used to lysis the red blood cell at room temperature for 10 min. After centrifuging twice at 250 g for 5 min, the precipitate was resuspended with 2 mL PBS (pH= 7.4). The sample was analyzed by a cytoflex flow cytometry and Kaluza 2.1 software was used to analyze the results.

Statistical data analysis

The data were represented as means±standards deviations. One-way analysis of variance (ANOVA) in SPSS 22.0 software package (SPSS Inc., USA) was used to assess statistical significances between PRV-treated groups. All statistical analysis was performed by GraphPad Prism 6.0. A value of $p<0.05$ or $p<0.01$ was regarded as significant differences.

RESULTS AND DISCUSSION

The survival rates of PRV-HLJ strain in mice were shown in Fig 1. At 48-hour post-infection (hpi) with the PRV-HLJ strain, the mice generally showed typical clinical neurological symptoms, such as pruritus. Mortality occurred within 60-96 hpi (Fig 1).

As shown in Fig 2(A-B) and Fig 3, the $CD3^+$ and $CD3^+CD4^+$ T lymphocyte induced by PRV were determined by FCM method. The value of $CD3^+$ T lymphocyte in mice was significantly decreased after 24 hpi ($p<0.01$) and remained at a low level until 72 hpi. Then the value of $CD3^+$ T lymphocyte increased dramatically.

In addition, we detected the change of $CD8^+$ T lymphocyte caused by PRV. It can be seen from Fig 4 that the $CD8^+$ T lymphocyte was significantly lower after 24 hpi ($p<0.01$). The change of $CD8^+$ T lymphocyte value was coincided with the $CD4^+$ T lymphocyte. Furthermore, as shown in Table 1, the value of $CD4^+$/ $CD8^+$ was increased at 24 hpi.

In addition, B lymphocyte with $CD19^+$ receptor was detected by FCM. It can be seen from Fig 2-E and 5 that $CD19^+$ B lymphocyte at 24 hpi decreased significantly. The low volume of $CD19^+$B lymphocytes could be observed for the first 12 h ($p<0.01$). Then $CD19^+$ B lymphocyte increased significantly from 60 to 72 hpi. At 96 hpi, the volume of $CD19^+$ B lymphocyte was lower compared to the early infection state ($p<0.01$).

The changes of the peripheral blood T/B lymphocyte volume caused by PRV are presented in Table 1. Pigs are

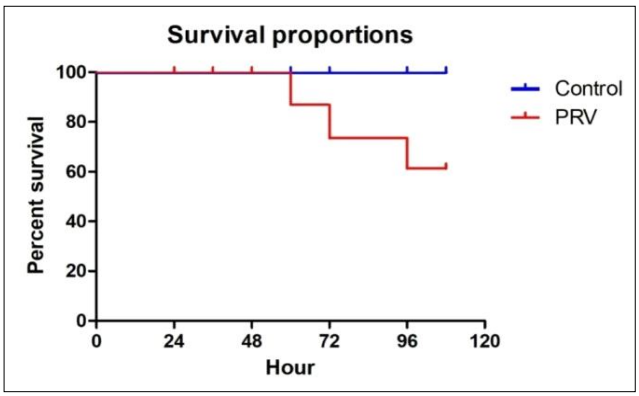
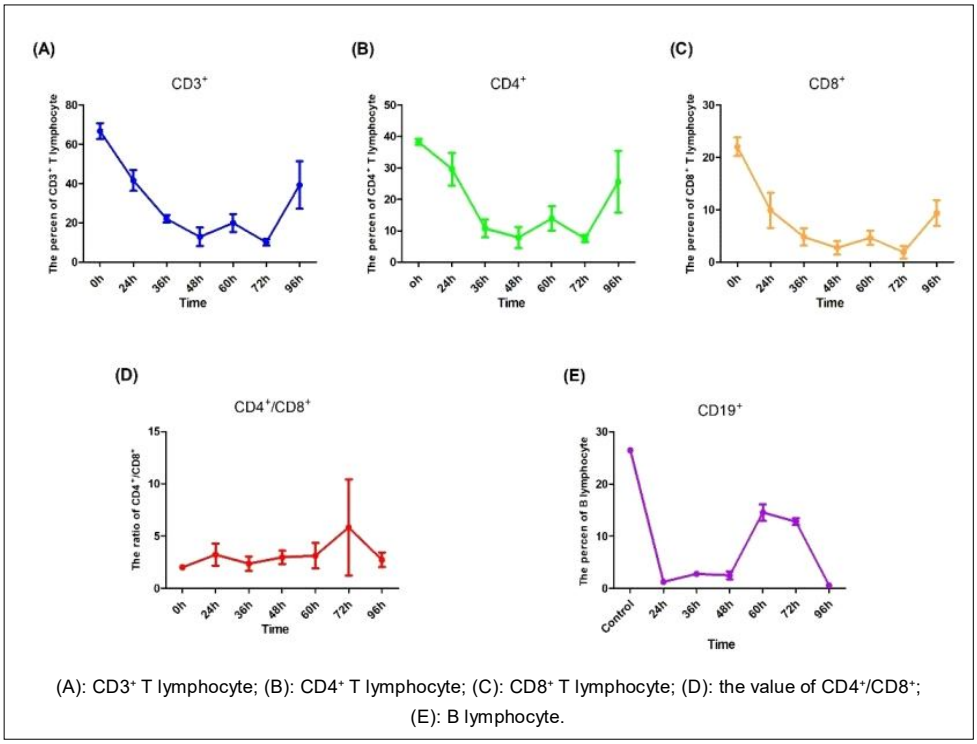


Fig 1: Survival proportions of control and PRV inoculated mice.

Table 1: The change of T/B lymphocyte induced by PRV.

Time	T lymphocyte				B lymphocyte
	$CD3^+$ (%)	$CD3^+CD4^+$ (%)	$CD3^+CD8^+$ (%)	$CD4^+/CD8^+$	$CD19^+$ (%)
0 h	66.77±4.06A	38.29±1.00Aa	22.06±1.76A	2.02±0.16b	26.50±0.50A
24 h	41.63±5.36B	29.63±5.15ABb	9.91±3.35B	3.23±1.05a	1.26±0.39CD
36 h	22.12±1.91a	10.74±2.83	4.84±1.64	2.37±0.69b	2.79±0.35C
48 h	12.93±4.68a	7.86±3.37	2.74±1.31	2.98±0.62b	2.52±0.75C
60 h	19.98±4.50a	13.89±3.88	4.65±1.38	3.13±1.22a	14.55±1.56B
72 h	10.11±1.63b	7.53±1.09	1.90±1.18	5.84±4.58a	12.82±0.67C
96 h	39.31±12.14B	25.61±9.84Bb	9.40±2.48B	2.75±0.70b	0.62±0.16D

Note: In the data of the same trade, different small letters indicated significant difference (p<0.05) and different capital letters indicated remarkably significant difference (p<0.01).



(A): $CD3^+$ T lymphocyte; (B): $CD4^+$ T lymphocyte; (C): $CD8^+$ T lymphocyte; (D): the value of $CD4^+/CD8^+$; (E): B lymphocyte.

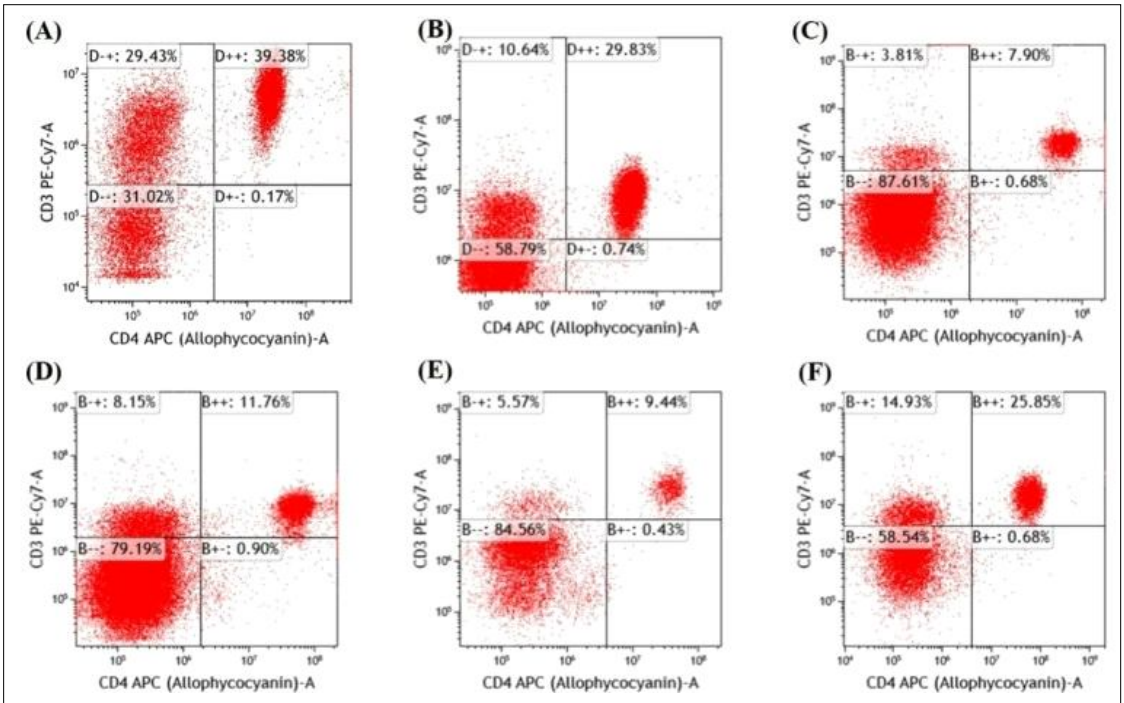
Fig 2: Dynamic detection of peripheral blood T/B lymphocyte in mice caused by PRV.

one of the major domestic animals that have an important role in food security and socio-economic starting point (Das *et al.*, 2022). AD caused by PRV is a common disease in many animals, especially in pigs (Zhao *et al.*, 2023). AD is a devastating pig disease and an important viral disease in pigs all over the world, which is characterized by high incidence rate in piglets and reproductive problems in sows and boars (Pegu *et al.*, 2016; Delva *et al.*, 2022). Only some strains of PRV cause severe respiratory disease in older pigs (Cun *et al.*, 2018). Although PRV elimination programs have been successfully implemented in domestic pigs in some countries (Müller *et al.*, 2011), infections frequently occur in the wild animals, especially in wild boars (Chiari *et al.*, 2015; Pedersen *et al.*, 2018). The uncontrollability of PRV in wild animals indicates a great potential risk for PRV transmission (Liu *et al.*, 2022).

In the past few years, scientists have conducted in-depth research on the immune mechanism during PRV infection. Previous clinical, immunological and virological data, strongly support the view of immunosuppression and viremia caused by PRV. According to that the virulence of PRV in pigs was related to that of mice (Minamiguchi *et al.*, 2019). Mice may be use as a model which can give some ideas for PRV kinetics (Tang *et al.*, 2017).

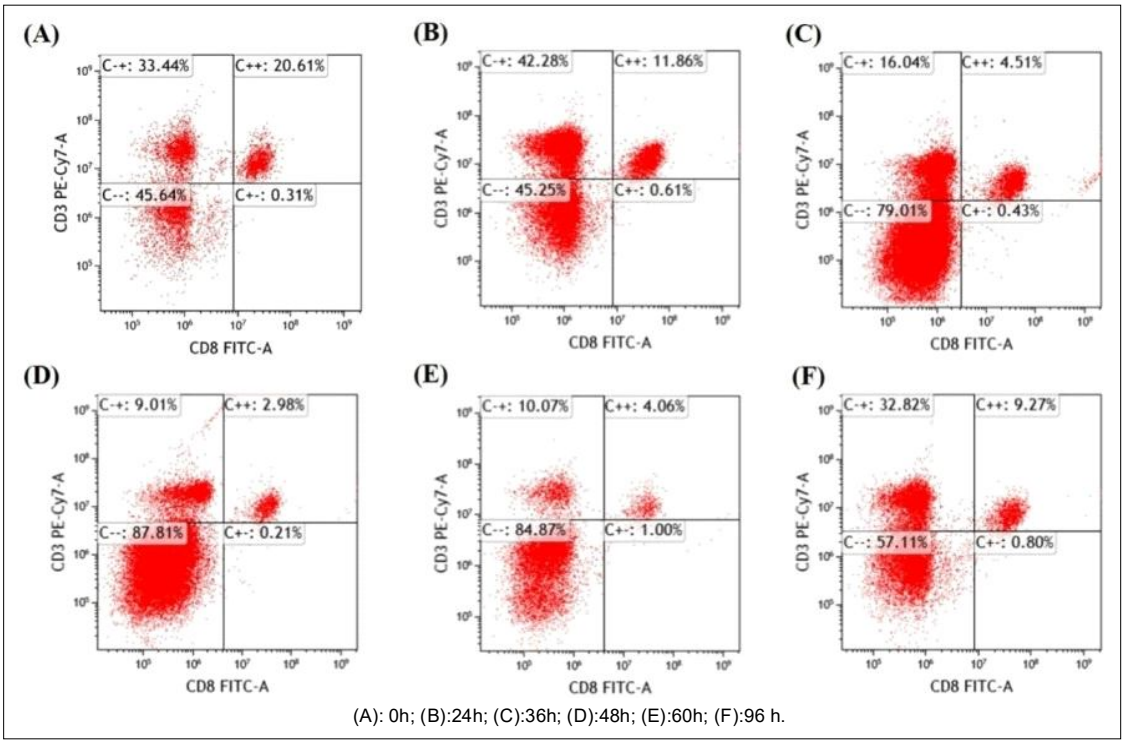
In this study, mice were infected with PRV and used to study the influence of the infection in the cellular adaptive immunity. The reference values of B and T cells and their sub-populations are very important to understand how the adaptive immune system is responding to virus infection. $CD4^+$ and $CD8^+$ T lymphocyte are the two kinds of T lymphocytes involved in cellular immunity (Arsenio, 2020). $CD4^+$ and $CD8^+$ T cells play an important role in the

recognition and activation by binding antigens to histocompatibility (MHC) II and I molecules, respectively (Lee and Jang, 2022). $CD8^+$ T cells play an important role in the adaptive immune response to intracellular viral infection. There is evidence indicating that $CD4^+$ $CD8^+$ T lymphocytes in peripheral blood have a pivotal role in preventing PRV infection (Ober *et al*., 1998). Previous study has shown that *in vitro* re-stimulation of peripheral blood lymphocytes of



(A): 0 h; (B): 24 h; (C): 36 h; (D): 48 h; (E): 60 h; (F): 96 h.

Fig 3: The FCM results of $CD3^+$and $CD3^+$ $CD4^+$ T lymphocyte induced by PRV.



(A): 0h; (B):24h; (C):36h; (D):48h; (E):60h; (F):96 h.

Fig 4: The FCM results of $CD3^+$ $CD8^+$ T lymphocyte caused by PRV.

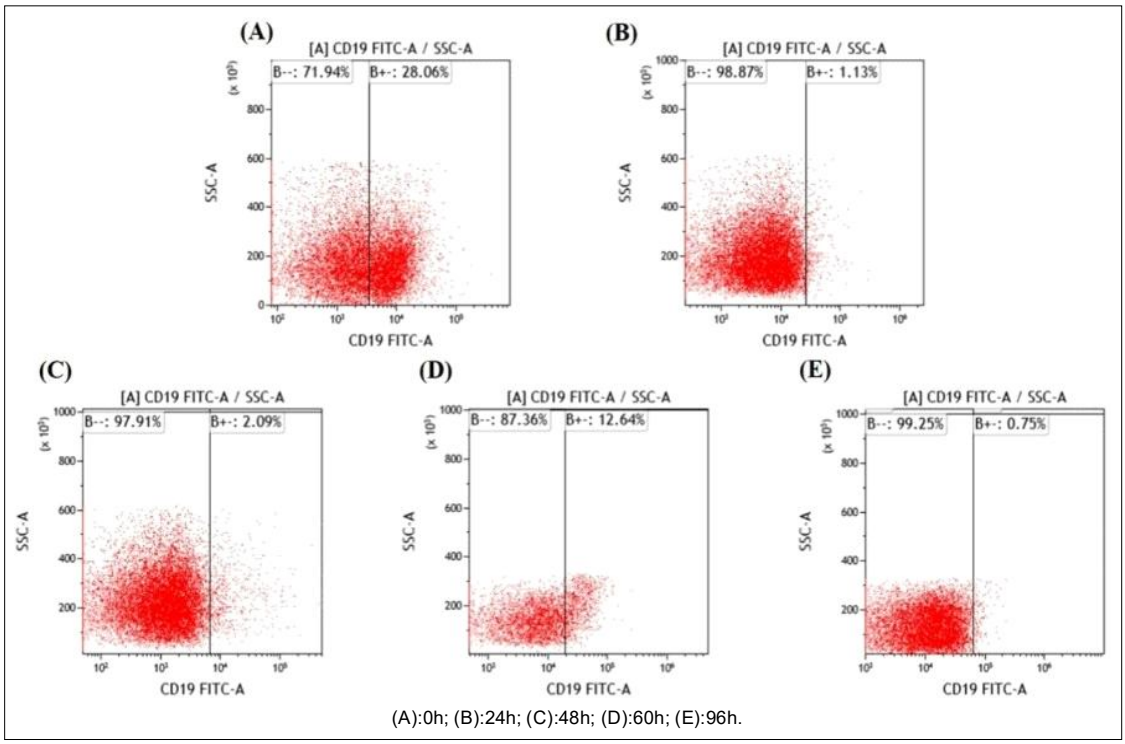


Fig 5: The FCM results of B lymphocyte (CD19$^+$) induced by PRV.

pigs infected with PRV induced a strong reaction of CD4$^+$CD8 α+ and CD4-CD8 α+ T cells (de Bruin *et al.*, 2000). In this study, we found that after PRV infection, the percentage of CD3$^+$ T cells, CD3$^+$CD4$^+$ T cells and the ratio of CD4$^+$/CD8$^+$ in mice peripheral blood could be decreased in a time-dependent manner. According to Page *et al.* (1992), animals with high CD8$^+$ T lymphocyte and low CD4$^+$/CD8$^+$ ratio can survive after PRV infection. In addition, animals with low CD8$^+$ T lymphocyte and high CD4$^+$/CD8$^+$ ratio developed severe disease and died. It maybe explains the mice death peak at 60-96 hpi PRV infection as shown in Fig 1.

CD19 molecule is a transmembrane glycoprotein of Ig superfamily, which is expressed by B cells in early pre-B phase and then is lost during plasma cell differentiation (Poe *et al.*, 2001). CD19 provides a B cell line specific component, a receptor complex containing widely expressed molecular (Sworder *et al.*, 2023). The density of CD19 on the cell surface was highly regulated during development and the expression levels of all mature conventional B cells in different peripheral lymphoid tissues were similar. In this work, we also investigated the dynamic change of CD19$^+$ B lymphocyte in the peripheral blood after PRV infection. The results showed that CD19$^+$ B lymphocyte decreased rapidly at 24 hpi and fluctuated until the end of this experiment.

CONCLUSION

This study was aimed to determine the dynamic change of T/B lymphocytes and their sub-populations in mice during PRV infection by flow cytometry. The results showed that PRV posed an immunosuppressive effect on the mice by decreasing the levels of B and T lymphocytes.

Authors' contributions

Qingyan Wang drafted the manuscript; Wei Sun and Samuel Kumi Okyere critically revised the manuscript; Shanshan Liu performed the animal experiment; Wei Sun and Qingyan Wang conceived this study and participated in the design of this work as well as data analysis. All authors gave final approval for publication and agreed to be responsible for the work performed.

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Conflict of interest

The authors declare no competing interests.

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