IMMUNOSUPPRESSIVE EFFECTS OF CYCLOPHOSPHAMIDE AND CORTICOSTEROIDS ON VIRUS GROWTH AND ANTIBODY RESPONSE TO A NON-PATHOGENIC STRAIN OF PSEUDORABIES VIRUS

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SUMMARY

Effects of cyclophosphamide (CPS) and corticosteroids (dexamethasone, DXM and flumethasone, FLM) on virus replication enhancement and antibody response to an apathogenic pseudorabies virus (PrV) strain, designated mA1p, were studied in mice. A significant ($p < 0.05$) increase of virus growth was observed in tissues of all drug-treated mice following primary inoculation of the virus. The replication, however, greatly decreased following secondary inoculation of the virus. The antibody (Ab) titres against the virus measured following drugs treatment, using an indirect ELISA technique, showed significant ($p<0.05$) decline in all drug-treated groups of mice. However, the highest decline was noted in CPS-treated mice followed by FLM and DXM-treated mice. No protection was observed in all groups of mice when they were challenged with lethal dose of the virulent virus.

Keywords: pseudorabies virus, antibody, immunosuppression, cyclophosphamide, corticosteroide

INTRODUCTION

Pseudorabies virus (PrV) is the causative agent of Aujeszky's disease in swine. The disease is characterized by inflammation of the respiratory tract and severe encephalomyelitis (Card *et al.*, 1990; Kluge *et al.*, 1992; Standish *et al.*, 1994). A Malaysian non-pathogenic PrV strain (mA1p) has been selected as a live vaccine against pseudorabies (Ali, 1999). The pathogenicity and immunogenicity of this clone virus in mice and pigs have been reported (Ali *et al.*, 1998; Ali, 1999).

Cyclophosphamide (CPS) is a non-selective, cytotoxic agent (Kolb *et al.*, 1977). The immunosuppressive effect CPS has been evaluated and reported to cause depletion in B-dependent lymphoid tissues and consequently decline in antibody response (Corrier *et al.*, 1979; Azmi, 1994; Fitzgerald *et al.*, 1996). In contrast, the immunosuppression induced by corticosteroides was particularly due to the depletion in the T-dependent lymphoid tissues lineage rather than B- cells (Shimizu and Shimizu, 1979; Narita *et al.*, 1985). Immunosuppressive effects of CPS to herpesvirus antigens such as equine herpesvirus–1 (EHV-1) have been reported (Field *et al.*, 1992). Immunosuppression induced by corticosteroides to herpes-simplex virus (HSV) (Tenser and Hsing, 1977), EHV-1 (Gibson, 1992) and bovine herpesvirus-1 (BHV-1) (Netleton and Sharp, 1980) was also reported.

This study was designed to determine the immunosupressive effects of cyclophosphamide and corticosteroides on virus replication and humoral immune responses to an apathogenic PrV strain. Consequently, the safety and effectiveness of the virus, selected as a live virus vaccine candidate against PrV infections, was confirmed.

MATERIALS AND METHODS

Viruses

The viruses used in the study were PrV-mA1p, a non-pathogenic clone of Malaysian PrV, established at virology Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia; PrV-CD, an American strain of PrV, which was kindly provided by Professor Anthony Castro from University of California-Davis, USA. The pathogenicity and immunogenicity of these viruses have been determined (Ali *et al.*, 1998).

Experimental Animals

Four-week-old, BALB/c, female mice, obtained from the Laboratory Animals Breeding Unit (UPM) were used.

Drugs

The drugs used in the study were cyclophosphamide monohydrate (Sigma, St. Louis, MO, USA) at a dose of 200 mg/kg body weight via s.c. injection; dexamethasone (9a- Fluoro- 16a- methyl- prednisolone) (Sigma, St. Louis, MO, USA) at a dose of 16 mg/kg body weight via s.c. injection, and Flumethasone (6a-Fluorodexamethasone) (Sigma, St. Louis, MO, USA) at a dose of 16 mg/kg body weight via s.c. injection also. The doses and administration of these drugs were as described by Field *et al.* (1992).

Viruses Preparation and Purification

Stocks of the above viruses were prepared by propagation in Vero cells cultures grown on Leibovitz's-15 (L-15) media (GIBCO BRL. Grand Island, USA) supplemented by antibiotic- antimycotic, anti-PPLO agents (GIBCO BRL. Grand Island, USA) and fetal calf serum (FCS) (GIBCO BRL. Grand Island, Paisely-Scotland). The viruses were then purified by sucrose gradient ultracentrifugation method as described by Ben-Porat *et al.* (1974).

Virus isolation from mice tissues

Groups of four mice were killed at days 3, 5 and 8 post-inoculation (p.i.) of the virus. Nasal turbinates, lungs, cerebra, cerebella and trigeminal ganglia were collected individually in 1ml ice-cold, serum-free L-15 medium containing 8% antibiotic-antimycotic and 2% anti-PPLO solutions. These tissues were homogenized with the electric homogenizer (Thyristor Regler, West Germany). The suspensions were subjected to sonic vibration for 1-2 min. in an ice cold sonic water bath and spun at 3,000 rpm for 10 min at 4°C in a refrigerated centrifuge (Hettich, Tuttingen, West Germany) to remove the cellular debris. The supernatants were then kept at -70°C before being titrated by means of plaques formation assays.

Virus titration by plaque assay

Ten-fold serial dilutions of the virus sample were made in serum-free L-15 medium. Hundred ml of each dilution were inoculated in duplicate onto a confluent monolayers of Vero cells grown on 24-wells tissue culture plate (two wells were inoculated with L-15 media only to act as negative controls). The virus was allowed to adsorb at 37°C for 45 minutes. Five hundred ml of L-15 medium containing 2% FCS, 1.2% carboxy-methoxy cellulose (CMC), 1% antibiotic-antimycotic and 1% anti-PPLO solutions was added to each well and the plate was incubated at 37°C. The plate was periodically monitored over 84-72 hours p.i. After 3-4 days incubation, the overlay medium was poured away and the monolayers were rinsed twice with PBS. Following this step, the cell monolayers were fixed for 10min with 70% methanol before being stained with 2.5% crystal violet solution for at least 10mins. The plate was then rinsed gently in tap water until plaques were clear for enumeration, dried at room temperature and plaques counted using an inverted-light microscope (OLYMPUS CK2, JAPAN). The virus titre was calculated as the mean number of plaques in the last dilution that showed plaques formation times the reciprocal of that dilution and expressed as $\log_{10}$ p.f.u. per sample. The limit of sensitivity was normally 10 p.f.u. per sample.

ELISA for detection of serum antibody

An indirect ELISA technique was developed to measure the Ab titres in mice sera. The technique employed was according to the principles of Voller *et al.* (1980), Clark and Barbara (1987), and Azmi and Field (1993) with some modifications. The test was carried out with a working volume of 50 ml for each reagent. The PrV-mA1p antigen was diluted in bicarbonate buffer to give a concentration of 10 mg per ml antigen protein. Each well of 96-well plate (Dynatech. Immulon, Virginia, USA) was coated with the antigen solution and incubated at 4°C overnight. The plate was then washed three times with phosphate-buffered saline Tween20 (PBST) using the automated microplate washer (Dynatech. MR 7000, USA). To block the non-specific binding, 2% of bovine serum albumin (BSA)-Fraction V (Sigma, UK) was added and the plate incubated at 45°C for two hours. The plate was washed three times as above. For the detection of serum antibodies, two-fold (or ten-fold) serial dilutions of test sera were added and the plate was incubated at 37°C for one hour. The plate was washed three times. Fifty ml of pre-diluted goat anti-mouse peroxidase conjugated immunoglobulin (Sigma, UK) was added and allowed to react with the antigen bound-mouse antibodies, by incubating the plate again at 37°C for one hour. The plate was washed three times. The 2,2'-Azino-bis (3-ethylbenzthioline-6-sulfonic acid) (ABTS) substrate (Sigma, UK) diluted in citrate-phosphate buffer (CPB) and supplemented with 0.01% of H_2O_2 was added and the plate was incubated at room temperature for 30-40 minutes. Upon completion of the reaction, the plate was read immediately in a spectrophotometer (Dynatech. MR7000, USA) at dual wavelength mode absorbance 410-490 nm. Hyperimmune and preimmune sera were included in the plate as positive and negative controls, respectively. End-point titres of serum Abs were determined by plotting the data, serum dilution against optical density (O.D). The Ab titre is equivalent to the $\log_{10}$ dilution of the serum that corresponds to an optical density value $\geq$ the mean of the wells of preimmune sera plus three times of the standard deviation.

Experimental design

To study the effect of the immunosuppressive agents on virus growth and Ab response to PrV-mA1p strain, two separate experiments were conducted:

Experiment I

The objective of this experiment is to study the effect of immunosuppressive agents on PrV-mA1p replication in mice tissues following primary inoculation of the virus. Four groups of 4-week-old, BALB/c, female mice (20 mouse per group), A, B, C and D were used in the study. Mice in group A were treated with CPS, those in group B mice with DXM, those in group C with FLM while those in group D were given PBS as control. The drugs were given subcutaneously starting 2 days before virus inoculation (with $1x10^6$ p.f.u. per mouse of PrV-mA1p intranasally until 21 days when all mice were challenged with 600 p.f.u. per mouse of PrV-CD intranasally also. Tissues were collected from mice at day 3, 5 and 8 p.i. for virus isolation.

Experiment II

The objective of this experiment is to study the effect of immunosuppressive agents on PrV-mA1p replication in mice tissues and antibody response following secondary inoculation of the virus. Four groups of 4-week-old, BALB/c, female mice (20 mice per group), A, B, C and D were used in the study. All mice were primarily inoculated with $1x10^6$ p.f.u. per mouse of PrV-mA1p intranasally. Three weeks later, mice in group A were given CPS, group B mice received DXM, group C mice received FLM and group D mice were given PBS as control. Two days later, all mice were re-inoculated with $1x10^6$ p.f.u. per mouse of PrV-mA1p intranasally also. The drug treatments in mice were continued for a further three weeks p.i. when all mice were challenged with 600 p.f.u. per mouse of PrV-CD i.n. Tissues were collected at day 3, 5 and 8 p.i. for virus isolation.

The blood was also sampled from all groups of mice at 5 days intervals throughout the period of the experiment and the sera were kept at –20°C before being tested using an indirect ELISA for Ab response.

Statistical Analysis

The statistical significance of differences between groups of data was determined using the two-tailed Student's unpaired t-test.

Table 1. The effect of immunosuppressive agents on PrV-mA1p replication in mice tissues following primary inoculation

Drug treatment	Days p.i.	Virus isolation				
		Nasal turbinate	Lung	Cerebrum	Cerebelum	Trigeminal ganglia
CPS	3	.65[a]±0.20*	3.02[a]±0.13	3.98[a]±0.22	3.64[a]±0.73	4.04[a]±0.73
	5	2.31[b]±0.23	1.71[c]±0.14	3.30[a]±0.25	3.19[a]±0.61	3.35[a]±0.54
	8	1.87[c]±0.26	1.91[c]±0.35	2.68[a]±0.40	1.97[c]±0.54	2.72[a]±0.62
DXM	3	2.18[b]±0.26	2.55[a]±0.24	3.51[a]±0.41	3.18[a]±0.47	3.38[a]±0.29
	5	2.11[b]±0.38	1.21[d]±0.32	2.81[a]±0.63	2.69[a]±0.91	2.81[a]±0.44
	8	1.55[c]±0.33	1.41[d]±0.29	2.76[a]±0.75	1.45[d]±0.68	2.24[b]±0.46
FLM	3	2.11[b]±0.48	2.43[b]±0.36	3.45[a]±0.68	3.06[a]±0.45	3.55[a]±0.61
	5	1.92[c]±0.20	1.14[d]±0.35	2.73[a]±0.51	2.45[b]±0.64	2.78[a]±0.68
	8	–	1.29[d]±0.30	1.62[c]±0.82	1.24[d]±0.38	2.06[b]±0.36
PBS	3	1.04[d]±0.24	1.45[d]±0.61	2.53[a]±0.47	2.10[b]±0.48	2.53[a]±0.52
	5	0.92[d]±0.47	–	1.62[c]±0.25	1.42[d]±0.91	1.65[c]±0.43
	8	–	–	0.42[d]±0.85	–	0.85[d]±0.58

* virus titre (geometric±s.d.); n= 4; - virus titre below the detection level (10 p.f.u. per organ)
CPS= cyclophosphamide, DXM= dexamethasone, FLM= flumethasone
[a, b, c, d] Means with the same superscripts do differ significantly ($p< 0.05$)

RESULTS

The effect of immunosuppressive agents on virus replication

The virus titres in mice tissues following primary and secondary inoculation of the virus are summarized in Tables 1 and 2, respectively. Following primary inoculation of the virus, significant ($p<0.05$) increase in virus growth in both respiratory and nervous tissues of mice was observed in all drug-treated groups of mice (as compared to the control group). The highest virus growth was observed in CPS treated mice followed by DXM and FLM treated mice. The virus was detected in mice tissues treated with the three drugs up to day 8 p.i. with various levels except for FLM treated mice where the nasal turbinates were found to be negative for the virus on day 8 p.i.(Table 1). Although the virus replication ability was increased following administration of the drugs, no mortalities and clinical signs were observed in all groups of mice. However, upon challenge of mice with the virulent virus, 100% mortalities were observed in mice in all groups except those treated with CPS which showed 75% mortalities.

Following secondary inoculation of the virus, low virus titres were detected in mice tissues. However, slightly and relatively higher (as compared to primary inoculation) virus growth was observed in CPS treated mice followed by DXM and FLM treated mice (Table 2). Following challenge of mice with the virulent virus, 100% mortalities were observed in mice in all groups.

The effect of immunosuppressive agents on antibody response

The effect of immunosuppressive agents on Ab response in mice to PrV-mA1p following secondary inoculation of the virus is demonstrated in Fig 1. Similar Ab responses were observed in mice all groups of mice before drug administration. Following the drug treatment, significant ($p<0.05$) decline in Ab titres was observed in mice in all groups (compared to the control group of mice). The highest decline in Ab response was

Table 2. The effect of immunosuppressive agents on PrV-mA1p replication in mice tissues following secondary inoculation

Drug treatment	Days p.i.	Virus isolation				
		Nasal turinate	Lung	Cerebrum	Cerebelum	Trigeminal ganglia
CPS	3	$0.94^{c}\pm0.28$*	$1.14^{b}\pm0.43$	$1.93^{a}\pm0.17$	$1.86^{a}\pm0.39$	$1.97^{a}\pm0.22$
	5	$0.84^{c}\pm0.61$	–	$1.38^{b}\pm0.43$	$1.29^{b}\pm0.22$	$1.41^{b}\pm0.29$
	8	–	–	$1.06^{b}\pm0.30$	–	$1.12^{b}\pm0.27$
DXM	3	$0.64^{c}\pm0.42$	$0.92^{c}\pm0.68$	$1.73^{a}\pm0.36$	$1.25^{b}\pm0.26$	$1.86^{a}\pm0.33$
	5	$0.59^{c}\pm0.37$	–	$1.09^{b}\pm0.27$	$1.09^{b}\pm0.48$	$1.22^{b}\pm0.42$
	8	–	–	$0.94^{c}\pm0.28$	–	$0.64^{c}\pm0.42$
FLM	3	$0.59^{c}\pm0.37$	$0.89^{c}\pm0.67$	$1.41^{b}\pm0.29$	$1.12^{b}\pm0.27$	$1.43^{b}\pm0.36$
	5	–	–	$1.14^{b}\pm0.43$	$0.92^{c}\pm0.68$	$1.09^{b}\pm0.48$
	8	–	–	–	–	$0.42^{d}\pm0.85$
PBS	3	–	–	$0.92^{c}\pm0.68$	$0.42^{d}\pm0.85$	$0.92^{c}\pm0.68$
	5	–	–	–	–	$0.42^{d}\pm0.85$
	8	–	–	–	–	–

* virus titre (geometric±s.d.); n= 4; - virus titre below the detection level (10 p.f.u. per organ)
CPS= cyclophosphamide, DXM= dexamethasone, FLM= flumethasone.
[a, b, c, d] Means with the same superscripts do differ significantly ($p< 0.05$)

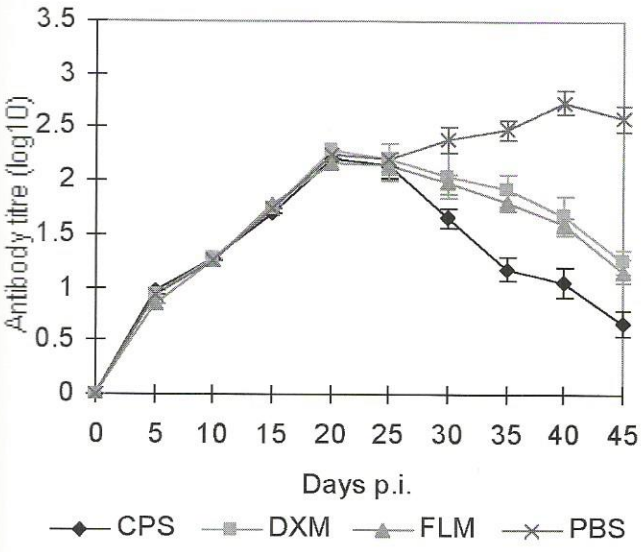

Fig. 1. The effect of immunosuppressive agents on antibody response following secondary immunization of mice with PrV-mA1p: Four groups (A, B, C and D) of 4-week-old, BALB/c, female mice (20 mice per group) inoculated with 1x 10^6 p.f.u. per mouse of PrV-mA1p i.n. (primary and secondary inoculaion) and treated with immunosuppressive agents beginning from day 21 p.i.. Data points represent ELISA antibody titres (geometric mean±s.d.; n=4).

Noted in mice treated with CPS, lower in DXM and FLM treated mice. On day 45 p.i., very low Ab titres in drug-treated mice were observed and compared to the control mice a decline of 2.12 $\log_{10}$, 1.34 $\log_{10}$ and 1.43 $\log_{10}$ in mice treated with CPS, DXM and FLM respectively, were noted.

DISCUSSION

Pseudorabies virus, mA1p strain, has been observed to be not pathogenic for mice and showed poor replication ability in mice tissues even when inoculated with very high doses (Ali *et al.*, 1998). It has also been proved to be not pathogenic for pigs (Ali, 1999), hence suggested as a live virus vaccine candidate against Aujeszky's disease. The present study provided further evidence that the virus was nonpathogenic in mice following immunosuppressive drugs administration. No mortalities or clinical signs were observed in mice even though there was enhancement of virus replication. This indicates that the virus can replicate in a manner that does not produce any damage to the host tissues or probably because the virus growth in the nervous tissues of mice is not luxariant to the extent that it arrests the cell functions.

The immune responses to the inducing antigens can usually be suppressed using the immunosuppressive agents. It was well established that CPS can non-specifically suppress B-lymphocytes functions (Corrier *et al.*, 1979; Azmi, 1994; Fitzgerald *et al.*, 1996) whereas corticosteroides suppresses T-lymphocytes functions (Narita *et al.*, 1985). In the present study, higher and consistent virus titres were isolated from mice tissues following primary inoculation of mice with PrV-mA1p and daily treatment with CPS. This indicates that an *in vivo* level of virus neutralization in this group of mice was not high since neutralizing Abs were crucial at early stages of viral infections, which substantiate the previous findings (Narita *et al.*, 1985). In contrast, lower virus titres were detected in tissues of mice treated with DXM and FLM (compared to CPS) to indicate that the effect of these corticosteroides on Ab level is mediated by a mechanism

Significantly ($p<0.05$) lower virus titres detected following secondary inoculation of PrV-mA1p (as compared to primary inoculation), was probably due to the higher Ab response evoked following virus replication in mice tissues after primary inoculation of the virus. The possibility of reactivation of latent virus after primary inoculation which evoke booster effect on the immune response may also considered a reason for the lower virus growth following the secondary inoculation of the virus (Mengeling, 1991). Following challenge of mice with the virulent virus, no protection was observed in either group following primary and secondary inoculation of the virus except for CPS treated mice where 25% protection was observed following primary inoculation. This indicates that Ab response, which is low in CPS-treated mice, does not the only factor in protection against PrV infections but cell-mediated immunity is, however, utmost important which was reported to be enhanced by CPS treatment while humoral immunity is declining (Corrier *et al.*, 1979).

Significant ($P< 0.05$) reduction in Ab levels in CPS treated mice (as compared to control mice) was also observed in the present study. This reduction was also noted in mice treated with DXM and FLM but is not as profound as that observed in CPS treated mice. This reflects the different mechanisms evoked by these drugs as it has previously been confirmed that CPS is not completely selective in its cytotoxicity between B- and T- lymphocytes populations. It is, however, particularly destructive to B-lymphocytes and their precursors (Burrows *et al.*, 1978). On the other hand, corticosteroides was particularly destructive for T lymphocytes rather than B- cells (Shimizu and Shimizu, 1979; Narita *et al.*, 1985). The effects of corticosteroides on Ab production is, therefore, confirmed the role of T-cells produced cytokines in Ab formation and production (Mosmann and Coffman, 1989; Romagnani, 1991).

Upon challenge of the drug-treated mice, Total mortalities were recorded in DXM and FLM-treated groups of mice after the primary inoculation of the virus and in all groups of mice after the secondary inoculation. Although a higher immunosuppression effect was observed in CPS treated mice, a certain degree of protection was obtained after challenge (25%) following primary inoculation of the virus. This indicates that Ab response, which is low in CPS treated mice, does not play a major role in the protection against PrV infection. However, CMI, especially the DTH response, is of utmost importance reportedly enhanced by CPS treatment while the humoral immunity was declining (Corrier *et al.*, 1979; Azmi, 1995). The most salient and significant conclusion emerging from this study is the safety of the Malaysian PrV strain (mA1p) when inoculated as a live virus in an immunosuppressed animal. The effects of different immunosuppressive agents on the immunogenicity of the virus was also revealed in the present study.

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REFERENCES

Ali, A.S., 1999. Immune responses to a live virus vaccine candidate and protection against pseudorabies virus infection. *Ph.D theis*, Universiti Putra Malaysia.

Ali, A.S., Mohd-Azmi, M.L. and Aini, I., 1998. Immune responses to non-pathogenic pseudorabies virus in mice. *Jurnal Veterinar Malaysia* **10**: 55-62.

Azmi, M. and Field, H.J., 1993. Interactions between equine herpesvirus type 1 and equine herpesvirus type 4: T cell responses in a murine infection model. *Journal of General Virology* **74**: 2339-2345.

Azmi, M., 1994. The immune responses to equine herpesvirus-1 (EHV-1) in a murine model. *Ph.D thesis,* University of Cambridge.

Azmi, M., 1995. Immunosuppressive effects induced by cyclophosphamide and cyclosporine A in mice infected with equine herpesvirus type-1 (EHV-1). *Jurnal Veterinar Malaysia,* **7**: 61-66.

Ben-Porat, T., De Marchi and Kaplan, A.S., 1974. Characterization of defective interfering viral particles present in a population of pseudorabies virions. *Virology* **61**: 29-37.

Burrows, P.D., Kearney, J.F. and Lawton, A.R., 1978. Pre-B cells: bone marrow persistence in anti-U- suppressed mice, conversion to B-lymphocytes, and recovery after destruction by cyclophosphamide. *Journal of Immunology* **20**: 1526-1531.

Card, J.P., Rinamman, L., Schwaber, J.S., Miselis, R. R., Whealy, M.E., Robbins, A.K. and Enquist, L.W., 1990. Neurotropic properties of pseudorabies virus:Uptake and transneural passage in the central nervous system. *Journal of Neuroscience* **10**: 1974-1994.

Clark, M.F. and Barbara, D.J., 1987. A method for the quantitative analysis of ELISA data. *Journal of Virological Methods* **15**: 213-222.

Corrier, D.E., Adams, L.G., Pierce, K.R., 1979. Effects of cyclophosphamide on the lymphoid tissues and humoral and cellular immune responsiveness of young calves. *American Journal of Veterinary Research* **40**: 1683-1689.

Field, H.J., Awan, A. R. and de la Fuente, R., 1992. Reinfection and reactivation of equine herpesvirus-1 in the mouse. *Archives of Virology* **123**: 409-419.

Fitzgerald, S.D., Williams, S.M. and Reed, W.M. 1996. Development of a chicken model for studying avian polymavirus infection. *Avian Diseases* **40**: 377-381.

Gibson, J.S., Slater, J.D., Awan, A.R. and Field, H.J., 1992. Pathogenesis of equine herpesvirus-1 in specific pathogen-free foals: primary and secondary infections and reactivation. *Archives of Virology* **123**: 351-366.

Kluge, J.P., Beran, G.W., Hill, H.T., and Platt, K.B., 1992. Pseudorabies (Aujeszky's Disease). In: Diseases of swine (Leman, A. D.; Straw, B. E.; Mengling, W.L.; D'Allaire, S. and Taylor, D.J., eds.), Ames, Iowa State University Press; 312-323.

Kolb, J.P.B., Poupon, M.F.M. and Lespinants, G.M. 1977., Splenic modifications induced by cyclophosphamide in C3H/He, nude and "B" mice. *Journal of Immunology* **118**: 1595-1599.

Mengeling, W.L., 1991. Virus reactivation in pigs latently infected with thymidine kinase negative vaccine strain of pseudorabies virus. *Archives of Virology* **120**: 57-70.

Mosmann, T.R. and Coffman, R.L., 1989. T_{H1} and T_{H2} cells: different patterns of lymphokine secretion lead to different functional properties. *Annual Review of Immunology* **7**: 145-172.

Narita, M., Haritani, M., Moriwaki, M. and Nanba, K., 1985. Pseudorabies virus in dexamethasone-treated pigs. *Veterinary Pathology* **22**: 417-419.

Nettleton, P.F. and Sharp, J.M., 1980. Infectious bovine rhinotracheitis virus excretion after vaccination. *Veterinary Records* **107**: 379-382.

Romagnani, S., 1991. Human T_{H1} and T_{H2} subsets: doubt no more. *Immunology Today* **12**: 256-257.

Shimizu, M. and Shimizu, Y., 1979. Effects of ambient temperature on clinical and immune responses of pigs infected with transmissible gastroenteritis virus. *Veterinary Microbiology* **4**: 109-116.

Standish, A., Enquist, L.W. and Schwaber, J.S., 1994. Innervation of the heart and its central medullary origin defined by viral tracing. *Science* **263**: 232-234.

Tenser, R.B. and Hsiung, G.D., 1977. Pathogenesis of latent herpes-simplex virus infection of the trigeminal ganglion in guinea pigs: Effects of age, passive immunization and hydrocortisone. *Infection and Immunity* **16**: 69-74.

Voller, A., Bidwell, D. and Barlett, A., 1980. Enzyme-linked imunosorbent assay in research. In *manual of Clinical Immunology*. Edited by H. Friedman. American Society of Microbiology. pp.359-371.

RINGKASAN

KESAN TEKANAN SIKLIFOSFAMID DAN KORTIKOSTEROID KE ATAS PERTUMBUHAN VIRUS DAN GERAKBALAS ANTIBODI TERHADAP STRAIN VIRUS PSEUDORABIES BUKAN PATOGEN

Kesan siklofofamid (CPS) dan kortikosteroid (dexamethasone, DXM and flumethasone, FLM) ke atas peningkatan pemreplikatan virus dan gerakbalas antibody terhadap strain virus pseudorabies (PrV) bukan pathogen, dinamakan mA1p, dikaji pada mencit. Kenaikan bererti ($p < 0.05$) pertumbuhan virus di tinjau pada tisu semua mencit yang diberikan drug selepas penginkulatan primer virus. Pemreplikatan sangat berkurangan selepas penginokulatan sekunder virus. Titer antibody terhadap virus di ukur seteleah rawatan drug, dengan menggunakan teknik ELISA, menunjukkan penurunan bererti ($p<0.05$) pada semua tikus yang dirawat dengan drug. Penurunan tertinggi di tunjukkan pada mencit yang dirawat dengan CPS dan dikuti dengan mencit yang dirawat dengan FLM dan DXM. Tiada perlindungan di tinjau pada semua kumpulan mencit apabila mereka dicabar dengan virus virulen pada tahap dos kematian .