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THE IMMUNOMODULATORY EFFECTS OF ALGAMMULIN ADJUVANT ON PROTECTION AND ANTIBODY RESPONSE TO PSEUDORABIES VIRUS

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SUMMARY

The potential of algammulin as adjuvant in the immune response to pseudorabies virus (PrV) was studied in mice. The antibody responses and protection against challenge were determined. A significant increase ($p<0.05$) in the protection levels in mice immunised with at least 10^3 p.f.u. per mouse of algammulin-adjuvant PrV antigens were observed compared to those immunised with the PrV antigen without adjuvant. Total protection was obtained following immunisation of mice with 10^6 p.f.u. per mouse. Following immunisation of mice, antibody response was greater ($p<0.05$) in mice immunised with the PrV antigen with adjuvant than in those immunised with the antigen alone. Booster dose using the algammulin-adjuvant PrV antigens slightly increased the antibody response. The antibody levels, however, were highly dose dependent.

Keywords: Algammulin, pseudorabies virus, protection, antibody

INTRODUCTION

Algammulin is an insoluble, polymorphic, non-toxic, non-pyrogenic, non-antigenic and active component of poly-fructose inulin with an anti-tumour activity in mouse, dogs, sheep, horses and donkeys (Cooper and Carter, 1986b; Cooper *et al*, 1994). It is an immune stimulant with strong vaccine adjuvant activity for both humoral and cell- mediated immune responses (Cooper and Steele, 1988). It specifically activates the alternative pathway of complement (Cooper and Steele, 1988) that plays the central role in the humoral immune responses (Schreiber and Muller-Eberhad, 1980) and cellular immune responses (Griffin, 1977; Schorlemmer, 1981; Sundsmo, 1982; Ross and Medoff, 1985). It is also a modifier of the natural immunity (Griffin, 1977).

Several studies on the adjuvant potential of this preparation to viral and non-viral antigens had been made. It had been demonstrated that 50% protection was obtained in mice immunised with the algammulin-adjuvanated influenza virus antigens. In contrast, no protection was obtained when mice were immunised with the same dose of non-adjuvanated virus antigens (Cooper and Steele, 1988). It had also been proved that algammulin enhanced the immunogenicity of hepatitis B surface antigen by 3- to 5.6 fold greater than that from the same antigen dose injected alone, and by up to 17 fold for the antigen keyhole limpet haemcyanin (KLH) in mice (Cooper *et al.*, 1991a,b). The potential of algammulin as an adjuvant preparation to the helminth parasites had also been reported (Chevis, 1990).

The effects of algammulin adjuvant on immunity to pseudorabies virus (PrV) had not yet been elucidated. In the present study we report the effect of algammulin adjuvant on the immune response to pseudorabies virus (PrV) in mice.

MATERIALS AND METHODS

Animals

Four-weeks-old, BALB/c female mice obtained from Laboratory Animals Breeding Unit, Faculty of Veterinary Medicine, Universiti Putra Malaysia were used in the study.

Viruses

Two isolates of pseudorabies virus were used:

(*i*) PrV-mA1p: A clone of a Malaysian local isolate (PrV-A1) established at the Virology Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia. This virus was found to be non-pathogenic for mice (Ali *et al.*, 1998) and was used as an immunising virus.

(*ii*) PrV-CD: An American exogenous virulent isolate kindly provided by Professor Anthony Castro from University of California-Davis (USA). This virus was proved to be highly pathogenic for mice (Ali *et al.*, 1998) and was used to challenged the immunised mice.

Adjuvant

Algammulin adjuvant is established at the ANUTECH company (Anutech court, Canberra ACT, Australia) and kindly provided by Dr. Ruth Sandeman from National University of Australia. Algammulin was used with the dose of 0.5 mg per mouse.

Virus preparation and purification

Viruses were propagated in Vero cells monolayers grown in Leibovitz's (L-15) medium (GIBCO BRL, Grand Island, USA) supplemented with 5% foetal bovine serum (FBS)(V/V) (GIBCO BRL, Paisley-Scotland), 1% antibiotic-antimycotic (V/V) and 1% anti-PPLO agent (V/V) (GIBCO BRL, Grand Island, USA). The viruses were purified from Vero cell cultures using sucrose gradient ultra-centrifugation as described by Ben-Porat *et al.* (1974) and the purity of viruses material was examined by electron microscopic examination.

ELISA for detection of serum antibody

An indirect ELISA technique was developed to detect the antibody (Ab) response in mouse sera based on well-established principles and protocols (Voller *et al*, 1980; Clark and Barbara, 1987; Ali and Mohd-Azmi, 1997) with some modifications. The test was carried out with a working volume of 50 µL for each reagent. Each well of 96-well plate (Dynatech. Immulon. Virginia, USA) were coated with PrV antigen solution diluted in bicarbonate buffer and incubated at 4°C overnight. The plate was then washed three times with phosphate-buffered saline Tween 20 (PBST) using an automated washing machine (Dynatech. MR 7000, USA). To block non-specific binding, 2% of bovine serum albumin (BSA-Fraction V) (Sigma, UK) was added and the plate was incubated at 45°C for 2 hours. The plate was washed again three times. Two-fold (or 10-fold) serial dilution of test sera were added and the plate was incubated at 37°C for one hour. The plate was washed three times. Pre-diluted goat anti-mouse peroxidase-conjugated immunoglobulin (Sigma, UK) was added and the plate was incubated at 37°C for one hour. The plate was washed three times. The ABTS substrate (Sigma, UK) supplemented with 0.01% of 30% H_2O_2 was added and the plate was incubated for 30-40 minutes at room temperature. The plate was read immediately on completion of the reaction in spectrophotometer (Dynatech, MR 7000, USA) at dual wave-length mode absorbance 410-490 nm. The end-point titres were determined by plotting ELISA data, serum dilution against the optical density. This enabled the value of $\log_{10}$ dilution to be read from the curve which corresponded to an optical density ≥ the mean of eight wells of preimmune sera plus three times the standard deviation. Hyperimmune and preimmune sera were used as positive and negative control respectively.

Experimental design

Three groups of mice (each group consists of seven subgroups of 8 mice) were used. The first group was immunised with the doses of 10^1- 10^6 p.f.u. per moue of PrV-mA1p without adjuvant. The second group was immunised with the same doses of adjuvanated PrV-mA1p. The third group was immunised as the second group but mice were boostered 3 weeks after the primary immunisation. The seventh subgroup in each group of mice was not given any inoculum as control (negative control). Another small group of 8 mice inoculated with the adjuvant only was also included in the experiment (reagent control). All mice were inoculated subcutaneously (s.c.) with an inoculum volume of 200 µL. Two weeks after immunisation all mice were challenged with 600 p.f.u. per mouse (2 LD_{50}) of PrV-CD via intranasal (i.n) route. Mortalities were recorded and the clinical signs were observed.

Statistical analysis

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

RESULTS

Protection in mice immunised with algammulin-adjuvanated PrV antigens

The protection levels obtained following immunisation of mice with algammulin-adjuvanated and non-adjuvanated PrV antigens were demonstrated in Fig. 1. Similar protection levels were obtained in all groups of mice immunised with ≤ 10^2 p.f.u. per mouse. For all groups of mice, The protection obtained with the dose 10^2 p.f.u. per mouse was low (37.5%) and with the dose 10 p.f.u. per mouse was much lower (12.5%). However, high protection levels were observed following immunisation of mice with at least 10^3 p.f.u. per mouse. Significant increase ($p < 0.05$) of protection in mice received adjuvanated PrV antigen (compared to non-adjuvanted antigen) was observed at the dose ≥ 10^3 p.f.u. per mouse. Boostering the mice with the adjuvanated PrV antigens significantly ($p < 0.05$) increased the protection levels only for the doses 10^3 and 10^4 p.f.u. per mouse as compared to primary immunisation. 100% protection was observed when mice were immunised with 10^6 p.f.u. per mouse of adjuvanated PrV antigens. In contrast, maximum protection obtained following immunisation of mice with the non-adjuvanated virus antigens was 87.5% at the same dose.

The classical signs of pseudorabies virus infection was clearly observed in non-immunised groups of mice (although they were transient and mice died quickly) but not in the immunised ones.

No protection was observed either in the non-immunised groups of mice (negative control) or in mice injected with the adjuvant alone (reagent control).

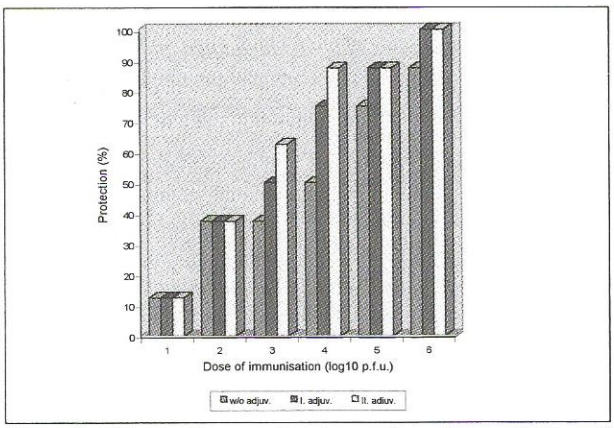

Fig. 1. Protection rate in mice immunised with algammulin adjuvanated PrV antigens

Antibody responses in mice immunised with algammulin-adjuvanated PrV antigens

The Ab responses following immunisation of mice with PrV were illustrated in Figs 2, 3 and 4. Generally, the Ab response elicited in all groups of mice was highly dose dependent. The Ab titres in the mice immunised with 10^4 p.f.u. per mouse of adjuvanated or non-adjuvanated PrV were relatively low whereas those in mice immunised with 10^5 p.f.u. per mouse were high and in ones immunised with 10^6 p.f.u. per mouse were much higher.

Following primary immunisation of mice, peak Ab titres were observed at day 30 post-immunisation in the mice immunised with 10^4, 10^5 and 10^6 p.f.u. per mouse of the non-adjuvanated virus antigens. In contrast, Ab titres in mice immunised with adjuvanated virus antigens were observed to peak on day 30 post-immunisation for the doses 10^4 and 10^5 p.f.u. per mouse and day 35 post-immunisation for the dose 10^6 p.f.u. per mouse (Figs 2 and 3).

Significant differences ($p < 0.05$) in Ab levels detected between the groups of mice immunised with the adjuvanated and non-adjuvanated PrV antigens were noted.

Following secondary immunisation of mice, peak Ab titres were observed on day 45 post-immunisation for the doses 10^4 and 10^5 p.f.u. per mouse and on day 50 for the dose 10^6 p.f.u. per mouse (Fig. 4). Also slight increases in the Ab titres after boosting the mice were observed. An increase of 0.22 $\log_{10}$, 0.13 $\log_{10}$ and 0.01 $\log_{10}$ was detected in the peak points for the doses 10^4, 10^5 and 10^6 p.f.u. per mouse, respectively.

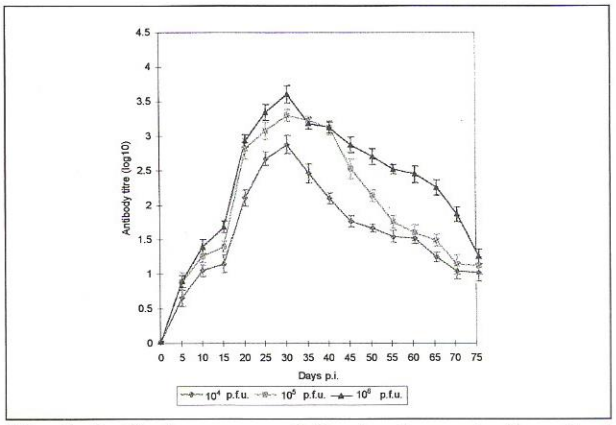

Fig. 2. Antibody response following immunisation of mice with non-adjuvanated PrV

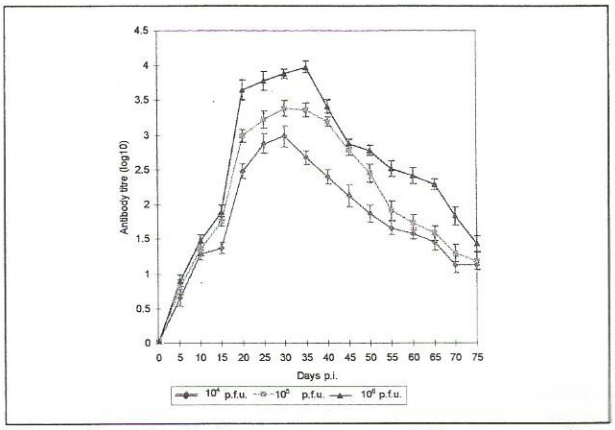

Fig. 3. Antibody response following primary immunisation of mice with algamulin-adjuvanated PrV

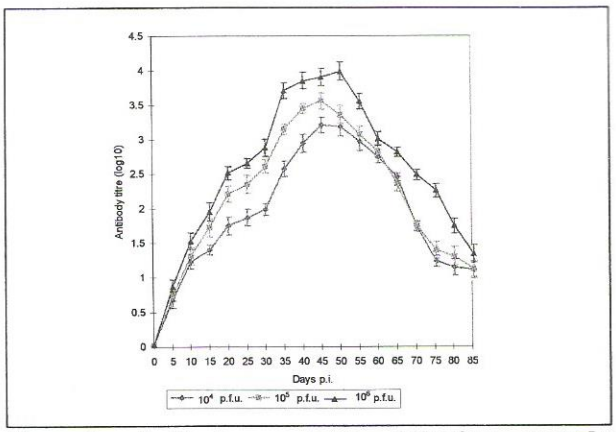

Fig. 4. Antibody response following secondary immunisation of mice with algamulin-adjuvanated PrV

Persistence of antibody response in the mouse sera for more than 2.5 month with >1.0 $\log_{10}$ was observed after boosting the mice with the adjuvanated virus antigens where an increase of 0.82 $\log_{10}$, compared to the primary immunisation, was noted for the immunisation dose 10^6 p.f.u. per mouse.

DISCUSSION

Adjuvants boost the immune responses in various ways. The ability of algammulin adjuvant for enhancement of immune responses to PrV has been demonstrated in the present study.

The findings obtained revealed that algammulin adjuvant has increased the protection levels in the mice immunised with PrV antigens and an increase in the antibody (Ab) response was also noted. However, the Ab response was not correlated with protection levels obtained as marked differences in protection levels and low differences in Ab response were observed among groups of mice immunised with the adjuvanted and non-adjuvanated antigens. This verify that the cellular immunity which is of utmost importance in protection against viral infections was also enhanced by algammulin adjuvant. This is found to be in agreement with Cooper *et al.* (1991b) who showed that algammulin boosts IgG, IgM, IgA responses as well as it activates T-cells responses.

Recently, we reported that total protection could not be obtained in mice immunised with 10^6 p.f.u. per mouse of PrV-mA1p (without adjuvant) following intranasal challenge (Ali *et al*, 1998). However, in the present study, total protection was obtained in the mice immunised with 10^6 p.f.u. per mouse of PrV adjuvanated antigens following intranasal challenge. This again indicates that the adjuvant enhanced more immune responses when incorporated with PrV antigens.

The Ab response to PrV was found to be highly dose dependent (whether the virus was incorporated with the adjuvant or not). This finding substantiate the previous results obtained by Ali and Mohd-Azmi (1997).

It was well established that algammulin adjuvant is a non-antigenic preparation by itself, however, it can potentially boost the immune response mechanisms when incorporated with the suitable antigens (Cooper and Carter, 1986a). In the present study, no protection was observed in mice given the adjuvant alone where total mortalities of mice were recorded.

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REFERENCES

Ali, A. S. and Mohd-Azmi, M. L. (1997). Measurement of antibody response to Aujeszky' disease virus in mouse sera by using an indirect ELISA. *In:* Proceedings of the 9th Veterinary Association Malaysia (VAM) Scientific Congress. Penang, Malaysia. 219- 222.

Ali, A. S., Mohd-Azmi, M. L. and Aini, I.(1998). Immune responses to non-pathogenic PrV (mA1p) in mice. *J. Vet. Malaysia.* **10**: 55-62.

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.

Chevis, R. (1990). *Ph.D Thesis. Australian National University.*

Clark, M. F. and Barbara, D. J. (1987). A method for the quantitative analysis of ELISA data. *J. Virol. Meth.* **15**: 213- 222.

Cooper, P. D. and Carter, M. (1986a). Anti-complementary action of polymorphic solubility forms of particulate inulin. *Molec. Immunol.* **23**: 895- 901.

Cooper, P. D. and Carter, M. (1986b). The anti-melanoma activity of inulin in mice. *Molec. Immunol.* **23**: 903- 908.

Cooper, P. D. and Steele, E. J. (1988). The adjuvanicity of gamma inulin. *Immunol. Cell Biol.* **66**: 345- 352.

Cooper, P. D., Turner, R. and Mc Govern, J. (1991a). Algammaulin (gamma inulin/alum hybrid adjuvant) has greater adjuvanicity than alum for hepatitis B surface antigen in mice. *Immunology Letters* **27**: 131- 134.

Cooper, P. D., McComb, C. and Steele, E. J. (1991b). The adjuvanicity of algammulin, a new vaccine adjuvant. *Vaccine* **9**: 408- 415.

Cooper, P. D., Steele, E. J., Mc Comb, C., Mc Govern, J. and Turner, R. (1994). Gamma inulin and algammulin: two new vaccine adjuvants, *In*: Recombinant and Synthetic Vaccines, Talwar, G. P., Rao, K. V. S. and Chanhan, V. S. (eds). Narosa Publishing House, New Delhi, India.

Griffin, F. M. (1977).*In*: Comprehensive Immunology, Vol. 2, Good, R. A. and Day, S. B. (eds), Plenum Press, New York, pp. 85- 113.

Ross, G. D. and Medoff, M. E. (1985). Membrane complement receptors specific for bound fragments of C3. *Adv. Immunol.* **37**: 217- 267.

Schorlemmer, H. U. (1981).The role of complement in the function of monocyte-macrophage system, *In*: Disorders of the Monocyte- Macrophage System, Schmalzl, F.; Huhn, D. and Schaefer, H. E. (eds), Springer-Verlag, New York, pp 59- 71.

Schreiber, R. D. and Muller-Eberhard, H. J. (1980). New developments in the activation of the alternative pathway of complement. *In*: Immunoassays: Clinical Laboratory Techniques for the 1980s, Nakamura, R. M., Dito, W. R. and Tucker, E. S. (eds), Alan, R. Liss, New York, pp 411- 431.

Sundsmo, J. S. (1982). The leukocyte complement system. Fed. Proc. **41**: 3094- 3098.

Voller, A. Bidwell, D. and Barlett, A. (1980). Enzyme- linked immunosorbent assay in research. *In*: Manual of Clinical Immunology. D.A. Friedman (ed). American Society of Microbiology. pp. 359- 371.

RINGKASAN

KESAN PENGIMUNOMODULATAN ADJUVAN ALGAMULIN DALAM PERLINDUNGAN DAN GERAKBALAS ANTIBODI TERHADAP VIRUS PSEUDORABIES

Potensi algamulin sebagai adjuvan dalam gerak balas imun terhadap virus pseudorabies (PrV) telah dikaji. Gerakbalas antibodi dan perlindungan terhadap cabaran telah ditentukan. Satu peningkatan tererti ($p<0.05$) dalam aras perlindungan pada mencit terimun dengan sekurang-kurangnya 10^3 p.f.u. per mencit mengguna antigen algamulin-adjuvan PrV telah dicerapkan berbanding dengan mencit terimun dengan antigen PrV tanpa adjuvan. Perlindungan sepenuh diperolehi berikutan pengimunan mencit dengan 10^6 p.f.u. per mencit. Berikutan pengimunan mencit ini, gerak balas antibodi lebih tinggi ($p<0.05$) dalam mencit terimun mengguna antigen PrV dengan adjuvan daripada yang terimun dengan antigen sahaja. Dos penokok mengguna antigen algamulin-adjuvan PrV meningkatkan sedikit gerak balas imun. Bagaimanapun, gerak balas antibodi paling bersandarkan dos.