

P.265

Influence of various adjuvants on antibody avidity and relative ratios of IgG2 to IgG1 (Th1 to Th2) in pigs after booster immunizations with SIV vaccines

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Introduction

Since no single adjuvant is universally applicable for all antigens, it is critical to include different types of adjuvant systems in an experimental vaccine for evaluation. In this study, an inactivated commercial swine influenza A (SIV) vaccine was used to evaluate the influence of five adjuvant systems on antibody avidity and the relative ratios of IgG2 to IgG1 (Th1 to Th2) in young pigs after booster immunizations.

Materials and Methods

Thirty six 21 day old pigs were assigned to six groups. Experimental vaccines were prepared by mixing the manufacturer's recommended amount of SIV antigens with one of five adjuvants supplied by MVP Laboratories, Inc. Pigs of group 1 through group 5 were given vaccines containing 20% Emulsigen®-D, 12% Emulsigen®/4% Rehydragel, 20% Emulsigen®-BCL, 20% Polygen™, and an adjuvant supplied by the manufacturer of the commercial SIV vaccine respectively. Pigs of group 6 were given PBS only. On day 0, all of the vaccinated pigs were given a 2 ml dose intramuscularly. On day 21, all pigs were given a second dose. All pigs were bled on day 0, day 21, and day 42. The hemagglutination inhibition (HI) test was conducted by the Veterinary Diagnostic Laboratory at Iowa State University. The antibody avidity of each pig serum was measured using a thiocyanate elution ELISA as described (1). IgG1 and IgG2 were determined in pig sera by ELISA as described (2).

Results and Discussion

Influence of adjuvant on relative ratios of IgG2 to IgG1 in pigs: Polygen™ induced the highest H1N1-specific and H3N2-specific IgG2 response (Table 1). This signals a Th1 response that leads to cell-mediated immunity. Furesz, et al, demonstrated that a high anti-hemolysin IgG2:IgG1 ratio correlated with hemolysin neutralization antibody titers and reduced lung lesion scores in *A. pleuropneumoniae* infected pigs (3). Emulsigen®-D and Emulsigen®/Rehydragel induced the highest levels of H1N1-specific and H3N2-specific IgG1. This indicates a Th2 response that leads to humoral immunity which is of importance in SIV infections.

Influence of adjuvant on antibody avidity: There is a significant difference between the SIV-specific antibody avidity indices (GMAI) of the five adjuvant groups with Emulsigen®-D producing the highest results (Table 2). It has been reported that higher serum avidity indices indicate more effective bactericidal activity of the microphages (2). This study indicates that the measurement of ratios of IgG2 to IgG1 and GMAI are useful as supplementary methods for predicting the properties of each test adjuvant in terms of their tendency to direct cellular (Th1) or humoral (Th2) immune response and antibody functional activity. These findings suggest that Th1 or Th2 responses in pigs are determined by both the adjuvant and its co-injected antigen.

Table 1. Adjuvant effects on relative ratios of H1N1-specific IgG2 to IgG1 and H3N2-specific IgG2 to IgG1.

| Vaccine adjuvant | H1N1-specific | | | H3N2-specific | | |
|-----------------------|-----------------|----------|------------------|-----------------|----------|------------------|
| | IgG2:IgG1 ratio | HI titer | Response profile | IgG2:IgG1 ratio | HI titer | Response profile |
| Emulsigen®-D | 0.81 | 1973 | Th2 | 0.74 | 2133 | Th2 |
| Emulsigen®/Rehydragel | 0.73 | 347 | Th2 | 0.81 | 200 | Th2 |
| Emulsigen®-BCL | 1.09 | 987 | Th1 | 0.92 | 480 | Th2 |
| Polygen™ | 1.24 | 147 | Th1 | 1.05 | 67 | Th1 |
| Control adjuvant* | 1.00 | 427 | Th1 | 0.81 | 280 | Th2 |

* Control adjuvant was obtained from the commercial vaccine used in this study.

Table 2. Adjuvant effects on the geometric mean avidity indices (GMAI) of anti-SIV IgG antibodies in pigs after booster immunization.

| | Adjuvant | GMAI (Range) | Statistical difference between Adjuvant (p value) |
|----|-----------------------|---------------------|---|
| 1. | Emulsigen®-D | 1.360 (1.200-1.400) | |
| 2. | Emulsigen®/Rehydragel | 1.100 (0.900-1.400) | p=0.0101 (1 vs 2)* |
| 3. | Emulsigen®-BCL | 0.970 (0.400-1.400) | p=0.0331 (1 vs 3)* |
| 4. | Polygen™ | 0.800 (0.600-1.000) | p=0.0001 (1 vs 4)* |
| 5. | Control adjuvant | 1.000 (0.900-1.100) | p=0.0101 (1 vs 5)* |

*Statistically significant difference at p<0.05.

References

1. Pullen, G. R., Fitzgerald, and C. S. Hosking. 1986. Antibody avidity determination by ELISA using thiocyanate elution. *J. Immunol Methods*. 86(1):83-87.
2. Vermont, C. L., van Dijken, H. H., van Limpt, C. J. P., de Groot, R., van Alphen, L., and G. P. J. M. van den Dobbelaars. 2002. Antibody avidity and immunoglobulin G isotype distribution following immunization with a monovalent Meningococcal B outer membrane vesicle vaccine. *Infect. Immun.* 70:584-590.
3. Furesz, S. E., Wilkie, B. N., Mallard, B. A., Rosendal, S., and J. I. MacInnes. 1998. Anti-haemolysin IgG1 to IgG2 ratios correlate with haemolysin neutralization titres and lung lesion scores in *Actinobacillus pleuropneumoniae* infected pigs. *Vaccine*. 16 (20):1971-1975.