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Host Immune Responses Against Hog Cholera Virus in Pigs Treated with an Ionized Alkali Mineral Complex

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Abstract

To determine the immune responses in pigs to hog cholera virus after treatment with an ionized alkali mineral complex (IAMC), 40 healthy pigs (28-32 days old) from a commercial swine farm were purchased and housed into 4 groups (n=10 each). All pigs were vaccinated intramuscularly (1 ml) with an attenuated live hog cholera virus (HCV, LOM strain) at 28-32 days old and challenged with a virulent hog cholera virus at 8 weeks after vaccination. Each group was treated with PowerFeelTMsprayed diet as 0.05% (w/w) in a final concentration (T-1, n=10), a diet mixed with SuperFeedTMas 3% (w/w) in a final concentration (T-2, n=10), or a diluted PowerFeelTMsolution (1:500, v/v) as drinking water (T-3, n=10), respectively. A group (n=10) served as a non-treated control. Proportions of expressing CD2+ and CD8+ cells increased significantly ($p<0.05$) at 8-week post-application. Mean antibody titers of each group against HCV gradually increased to higher levels after vaccination and with challenge of the virulent virus. In conclusion, the IAMC-treated diets can be helpful for the improvement of growth in pigs with proper vaccination program, while the IAMC-treated diets have no effects on the clinical protection against hog cholera.

Key words : Ionized alkali mineral complex, Hog cholera virus, Porcine immune cells.

Introduction

Hog cholera, so called classical swine fever, is an acute infection manifested by high fever, depression, anorexia and conjunctivitis [3]. After that, nervous system dysfunctions, a diffuse hyperemia and purplish discoloration of the light skin are exhibited [13]. In Korea the disease has been one of the major diseases that are threatening the expanding Korean swine industry since 1947 [7]. Thus, the national eradication program of a virulent hog cholera virus infection in the industry is of major veterinary importance. Protective immunity of an attenuated live hog cholera virus (LOM strain) vaccine has been well approved in Korea through the establishment of solid serum-neutralizing antibody [6]. Under the sporadic occurrence of the disease annually, therefore, national mass-vaccination program by the government has suggested the first vaccination at 40 days old and the second vaccination at 60 days old, with annually booster injection for adults. Since December, 2001 the Korean government has ceased vaccination policy against hog cholera.

As a nonspecific immunostimulator, ionized alkali mineral complex (IAMC) which consists of Si, Ag, Na and K ions, has been applied for the improvement of swine growth [Y.H. Park et al. 1998. Proceed 15th Int. Pig Vet. Soc., Birmingham, England, p22]. Immunostimulatory effects on pigs were demonstrated through proliferation and activation of porcine immune cells [9, 14]. However, the effects to host animals and practical mechanisms are of controversy.

Thus, the objective of this study was to determine the host immune responses to a virulent hog cholera virus in pigs vaccinated with a dose of an attenuated live hog cholera virus vaccine with an IAMC treatment.

Materials and Methods

Ionized alkali mineral complex (IAMC)

PowerFeelTM and SuperFeedTM were kindly supplied by NEL Biotech Co., Ltd. (Ansung, Korea).

Animals and treatments

Forty healthy pigs (28-32 days old) from a commercial swine farm were purchased and housed into 4 groups at swine pens of the College Experiment Station. Each group (n=10) was treated with a diet described previously [9]. In brief, pigs of T-1 were treated by a basic diet sprayed with

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PowerFeelTMsolution as 0.05% (w/w) in a final concentration and those of T-2 were treated with a basic diet mixed with SuperFeedTMas 3% (w/w) in a final concentration. Pigs of T-3 were treated with a diluted PowerFeelTM solution (1:500, v/v) as drinking water and control pigs were treated with a basic diet and tap water.

Isolation of leukocytes and monoclonal antibodies

Peripheral bloods were collected from pigs at pre-application, 5-, 8- and 12-weeks post-application (PA) of the IAMC, respectively and leukocytes were separated by a method described in a previous report [9]. Six monoclonal antibodies reactive to porcine leukocyte differentiation antigens, H42A, MSA4, PT90A, PT81B, Pig45A and PT79A [9] were used for a flow cytometry (FACSCalbur, Becton Dickinson Immunocytometry Systems, San Jose, CA, U.S.A.), and acquired data were then analyzed with a Cell Quest program (Becton Dickinson, version 3.1f). The percentages of lymphocytes with epitopes to the various antibodies were calculated.

Hog cholera virus and clinical observations

All pigs were vaccinated intramuscularly (1 ml) with an attenuated live hog cholera virus (LOM strain) at 28-32 days old and challenged intranasally with a virulent hog cholera virus (2 ml, 1040TCID50/ml) at 8-weeks post-vaccination. Clinical observations were performed daily for 4 weeks after challenge.

Serology

Sera were collected at the same intervals from peripheral bloods and hog cholera virus specific antibodies were detected by an indirect immunofluorescent assay (IFA) [16]. For the IFA test, PK-15 cell monolayers infected with hog cholera virus (LOM strain) were prepared in 96-well test plates. The 0.2 ml of the cell suspension (1×105 cell/ml) was transferred to each well of 96-well plates and incubated for 24 hours at 37℃. The monolayers were washed 3 times with phosphate buffered saline (PBS, pH7.4) and 0.2 ml of the virus (1030 TCID50/ml) was transferred to each well. This was incubated at 37℃ for 72 hours and then the medium in the plates was replaced by a cold mixture of 5% acetone in absolute ethanol (0.1 ml/well). The plates were stored at -20℃ until use. Negative and positive control sera were included in each test. IgG IFA test using commercial antiswine IgG fluorescein isothiocyanate conjugate (Kirkegaard & Perry Laboratories, Gaithersburg, MD, USA) were performed as previously described [15].

Statistical analysis

The Student's *t* test was used to compare the mean values obtained from three groups. One way analysis was performed with the mean values from three treated groups against that of control. Data were expressed as mean ±SD.

Results

Proportional comparison of porcine leukocyte subpopulations in pigs treated with non-specific immunomodulators was summarized in Table 1. As shown in the table, proportions of some subpopulation in pigs were variable before IAMC application. Proportion of expressing MHC-class II decreased at 5-weeks post-application in T-1 group and at 8-weeks post-application in T-2 pigs, while that increased at 4-weeks after challenge in all three treated groups, when compared to non-treated control group. However, there was no significant difference between those of all three treated groups and that of control. In the proportions of T lymphocyte (CD2+) against that of control group increased significantly at 8 weeks post-application in T-1 and T-2 groups ($p<0.05$). Proportion of expressing PoCD4+ increased only in T-3 group against control at 8-weeks post-application. In proportions of expressing PoCD8+, T-1, T-2 and T-3 had significantly higher mean values at 8-weeks post-application ($p<0.05$), while all treated groups had lower values after challenge when compared to control. The proportion of surface IgM+B lymphocytes against that of control decreased with significant change for T-1 at 5-weeks post-application. In the proportions of N cells against that of control, there were no significant changes.

Antibody titers against hog cholera virus (HCV) were measured through the detection of HCV-specific antibodies (Tables 2). Before vaccination pigs were variable in the level of maternal antibody titers (<1:4~1:256). Mean antibody titers of each group against HCV increased gradually after the vaccination of the attenuated live hog cholera vaccine (LOM strain) virus to higher levels with challenge of the virulent hog cholera virus. The distribution of titers was 1:16~1:256 at 5-weeks postvaccination and 1:16~1:1,024 at 8-weeks postvaccination. The humoral immune responses were increased dramatically by a virulent hog cholera virus (the titers 1:256~1:4,096). Clinical observations after challenge infection revealed high fever, depression, anorexia, conjunctivitis, diarrhea and purplish discoloration in a few pigs of all groups.

Discussion

Several studies have indicated that cell-mediated immunity is not a critical factor, but humoral immunity plays a major role in protection against hog cholera virus infection [1, 12]. The marked correlation between the titer of neutralizing antibodies and the protective effect after immunization with attenuated live hog cholera virus vaccine was approved. Therefore, humoral immune mechanisms are important host defense reactions in hog cholera virus infection [6]. Before vaccination pigs were variable in the level of antibody titers against hog cholera virus (<1:4~1:256). This kind of antibody might influence the vaccine efficacy, so mean antibody titers of each group against HCV increased gradually after the vaccination of the attenuated live hog

Table 1. Proportional comparison of porcine leukocyte subpopulations in pigs treated with ionized alkali mineral complex

	Weeks post-application			
Group	**0**	**5**	**8**	**12**
<MHC class Ⅱ cells>				
T-1	13.73±1.33*	15.56±6.25	23.80±5.95	16.03±4.72
T-2	12.20±3.01	17.86±6.61	17.46±4.18	17.54±4.55
T-3	15.50±2.29	17.05±5.90	26.51±3.55	18.37±6.31
Con	16.09±7.79	19.88±4.82	24.78±13.03	14.65±4.17
<CD2+ cells>				
T-1	77.28±4.98	67.03±7.18	78.48±5.24a	59.43±9.30
T-2	77.72±8.01	74.20±13.11	81.61±6.74a	59.70±16.18
T-3	83.10±0.00	71.08±9.84	74.91±11.46	62.60±5.92
Con	77.95±6.73	70.35±16.66	71.96±5.88	61.30±17.11
<CD4+ cells>				
T-1	25.47±4.05	23.84±6.25	29.44±4.97	24.20±5.54
T-2	26.12±8.80	27.80±9.05	28.60±10.89	27.86±12.47
T-3	37.30±7.35	22.52±6.86	34.47±5.45	27.87±4.45
Con	29.80±11.90	25.05±9.51	29.68±5.55	26.05±1.48
<CD8+ cells>				
T-1	38.18±7.95	46.81±11.77	45.19±13.52a	34.85±5.30
T-2	40.10±10.44	49.53±15.58	55.41±18.66a	34.12±10.79
T-3	40.05±10.25	43.32±12.37	41.99±12.02a	37.07±15.77
Con	39.30±13.03	44.80±13.27	31.06±7.46	40.85±1.10
<B cells>				
T-1	9.85±3.72	3.73±1.62a	14.56±3.41	11.20±1.85
T-2	10.66±3.40	7.85±3.32	15.73±6.20	12.40±5.81
T-3	12.05±0.49	12.18±6.30	25.61±6.49	16.14±11.67
Con	12.00±5.65	9.33±4.99	22.98±9.96	19.95±2.86
<N cells>				
T-1	18.25±4.61	17.87±4.56	17.17±3.23	23.00±2.05
T-2	18.78±6.42	20.13±6.30	19.04±3.35	23.43±7.49
T-3	18.70±3.68	24.25±6.69	28.28±6.70	21.40±8.84
Con	18.03±5.21	23.05±7.77	27.95±10.63	18.45±2.19

All pigs were vaccinated with 1 ml of attenuated live hog cholera virus (LOM strain) vaccine intramuscularly at 28-32 days old and challenged with a virulent hog cholera virus (2 ml, 104TCID50/ml).
T-1 : pigs treated with a basic diet sprayed with PowerFeelTM solution to be 0.05% (w/w) in a final concentration.
T-2 : pigs treated with a basic diet mixed with SuperFeedTM to be 3% (w/w) in a final concentration.
T-3 : pigs treated with a diluted PowerFeelTM solution (1:500, v/v) as drinking water.
Con; pigs supplied with a basic diet and tap water.
* : mean ± SD
a : significant difference against that of control ($p<0.05$).

Table 2. Indirect fluorescent antibody titers against hog cholera virus in pigs

Group	No of pig	Weeks post-application			
		0	5	8	12
T-1	10	<4 - 16	16 - 256	16 -64	256 - 4,096
		(0.50)*	(2.20)	(2.50)	(5.40)
T-2	10	< - 16	16 -256	16 -1,024	1,024 - 4,096
		(0.60)	(2.63)	(3.14)	(5.57)
T-3	10	<4 - 256	16 -256	64 -256	64 - 4,096
		(1.33)	(3.00)	(3.44)	(4.88)
Control	10	<4 - 64	16	16 - 256	256 - 4,096
		(1.11)	(2.00)	(3.00)	(5.38)

All pigs were vaccinated with 1 ml of modified live hog cholera virus (LOM strain) vaccine intramuscularly at 28-32 days old and challenged with a virulent hog cholera virus (2 ml, 104TCID50/ml) at 8-weeks post-application.
T-1 : pigs treated with a basic diet sprayed with PowerFeelTM solution as 0.05% (w/w) in a final concentration.
T-2 : pigs treated with a basic diet mixed with SuperFeedTM as 3% (w/w) in a final concentration.
T-3 : pigs treated with a diluted PowerFeelTM solution (1:500, v/v) as drinking water.
Con : pigs supplied with a basic diet and tap water.
* mean IFA titers (Log4x).

cholera virus (LOM strain). Therefore, under the sporadic occurrence of the disease, national mass-vaccination program ought to recommend two injections at 3-weeks interval.

A previous report suggested that the infection of lymphocytes contributes to the depletion in their numbers after infection and leads to defective antibody production during the infection of virulent classical swine fever virus [8]. However, the humoral immune responses increased dramatically by challenge of a virulent hog cholera virus (the titers 1:256~1:4,096). It seemed that the challenge hog cholera virus originated from chronic case of the disease might have the booster effect. By the way, the weak point of this study was that the pathogenicity of a virulent challenge virus was not confirmed in pigs after isolation from the chronic case of the disease. Again previous reports mentioned that a modified live hog cholera virus (LOM strain) vaccine has the pathogenicity like other virulent strains of hog cholera virus, while the virulence of the virus is much less than them [5, 11]. Further study remains to determine the viral characteristics among field viruses.

The host immune system could be elucidated using a panel of monoclonal antibodies specific to leukocyte differentiation molecules of animal species [2]. Along with severe decrease of leukocyte and lymphocyte counts, each number of MHC class II, CD1 CD2, CD4, CD8 antigen positive cells and CD4+CD8+ double positive cells and sIgM+ B cells decreased abruptly two days after inoculating virulent ALD strain of HCV. However, each count of subpopulations were not recovered during the time of experiment until death of pigs [5, 10]. In addition, in pigs vaccinated with attenuated live hog cholera virus, absolute numbers of leukocyte, lymphocyte and lymphocyte subpopulations with the exception of the null cells decreased transiently from 2 to 8 days after inoculation [4]. As shown in the Table 1, proportions of some subpopulation in pigs were variable before IAMC application, while the variability became constant after the treatment. An IAMC-treated pigs showed significant reduction of the lymphocyte subpopulations compared with those of control, suggesting that the virus replication and persistence in the leukocytes after hog cholera virus infection might be altered, resulting in the most important features in the pathogenesis of hog cholera in pigs. Pathogenic mimic of hog cholera virus in pigs treated with the IAMC should be further discussed on the viral pathogenicity with relation to the mechanism of antibody production.

Those of expressing MHC-class II showed significant increase at 4-weeks after challenge in T-2 pigs. However, in the proportions of T lymphocyte (CD2+) against those of control group significant increases were observed at 8-weeks post-application in T-1 and T-2 pigs, also at 4-weeks after challenge in T-1 and T-3 pigs. Those expressing PoCD4+ showed significant increase only for that of T-3 against that of control at 8-weeks post-application ($p<0.05$). In addition, in those expressing PoCD8+all three groups showed significantly higher mean values at 8-weeks post-application against those of control, whereas the change against T-3 group was significant for that of T-2 at 8-weeks post- application and for that of T-3 at 4-weeks after challenge ($p<0.05$). This result suggested that the cell-mediated immunity also plays a important role in hog cholera virus infection. Interestingly, pigs treated with IAMC have achieved a significant improvement compared to control pigs, but there was no difference on the clinical protection against a virulent strain of hog cholera virus among the groups (data not shown).

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