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Relation between lymphocyte subpopulations of peripheral blood and immune responses of modified live hog cholera virus vaccine in pigs treated with an ionized alkali mineral complex

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Thirty-nine healthy pigs (28-32 days old) were purchased from a commercial swine farm and housed at swine pens of the College. The animals were vaccinated intramuscularly (1 ml) with an attenuated live hog cholera virus (HCV, LOM strain) and then boostered at 5 weeks after the first vaccination. The animals were divided into 4 experimental groups: 0.05% (w/w) PowerFeel™-supplemented diet (T-1, n = 10); 3% (w/w) SuperFeed™-supplemented diet (T-2, n = 10); diluted PowerFeel™ solution (1 : 500, v/v) as drinking water (T-3, n=9); control (n=10). PowerFeel™ is an original form of ionized alkali mineral complex (IAMC) and SuperFeed™is a commercial product of IAMC. The subpopulation of lymphocyte in blood was assayed by a flow cytometry and HCV-specific antibody was determined by an indirect immunofluorescence assay. In IMAC-treated groups, the proportions of subpopulation expressing MHC-class II, $CD2^+$, $CD4^+$, $CD8^+$, and surface IgM^+ B lymphocytes were significantly decreased at 5-weeks after the first vaccination. Significant decreases were also observed in the proportions of MHC-class II, $CD2^+$ and $CD8^+$ lymphocyte at 3-weeks after the booster injection. The humoral immune responses in T-1 and T-2 groups were greater than those in T-3 or control group. These results suggest that IAMC-supplemented diets may have an HCV-specific immunostimulatory effect in pigs.

Key words: Ionized alkali mineral complex, lymphocyte subpopulations, attenuated hog cholera virus vaccine

Introduction

Since hog cholera in Korea has been first recognized by laboratory tests in 1947, it has been one of the major diseases threatening the expanding Korean swine industry [8]. The disease is an acute infection manifested by high fever, depression, anorexia, and conjunctivitis [4]. After two to six days of an incubation period, the dysfunctions of nervous system such as paresis, circling tremors and occasionally convulsions are followed and light skinned pigs exhibit a diffuse hyperemia and purplish discoloration of the skin especially on the ears, abdomen, inside of the hindlegs and flanks [4, 15]. Thus, in order to reduce the economic loss from a virulent hog cholera virus infection, an effective immunization method with a modified live hog cholera virus vaccine has been well approved in Korea [7]. A national mass vaccination program using the attenuated live hog cholera virus (LOM strain) vaccine, therefore, would be the best choice for reducing clinical outbreaks of hog cholera and be helpful for eradicating the disease under the endemic spread and the sporadic occurrence of the disease.

In addition, because of increasing demands for the improvement of swine production performance by disease control and for safe animal products without any residual antimicrobial reagents in animal tissues, an ionized alkali mineral complex (IAMC) was applied to pigs to improve host defensive system of newborn piglets [11] and growing pigs [10]. Therefore, the objectives of this study were to determine the relation between lymphocyte subpopulations of peripheral blood and immune responses of modified live hog cholera virus vaccine in pigs treated with an IAMC.

Materials and Methods

Ionized alkali mineral complex (IAMC)

PowerFeel™, which is a liquidized original form of IAMC, and an applicable product, SuperFeed™, which is a fermented rice bran after mixing with PowerFeel™ to be 1.5% (w/w) in a final concentration, were kindly supplied by NEL Biotech Co., Ltd.(Ansung, Korea).

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Animals and treatments

Thirty-nine healthy pigs (2832 days old) were purchased from a commercial swine farm and housed at swine pens of the College Experiment Station. They are divided into 4 experimental groups including 0.05% (w/w) PowerFeel™-supplemented diet (T-1, n = 10), 3% (w/w) SuperFeed™-supplemented diet (T-2, n = 10), diluted PowerFeel™ solution (1 : 500) as drinking water (T-3, n = 9), and untreated control (n = 10). The pigs were treated throughout the whole period of the experiment. Feeds and waters were taken *ad libitum*. Feed for each group was formulated from a local feedmill company as usual.

Isolation of leukocytes and monoclonal antibodies

Peripheral bloods were collected from pigs at pretreatment, 5- and 8-weeks post-application (PA) of IAMC, respectively and leukocytes were separated by the method described in a previous report [10]. Six monoclonal antibodies [10] were used for staining porcine lymphocyte subpopulations by a flow cytometry (FACSCalbur, Becton Dickinson Immunocytometry Systems, San Jose, CA, U.S.A.). Data were analyzed with a Cell Quest version 3.1f program (Becton Dickinson). The percentages of lymphocytes with epitopes to the various antibodies were obtained.

Hog cholera virus vaccination and serology

All pigs (28-32 days old) were vaccinated intramuscularly (1 ml) with an attenuated live hog cholera virus (LOM strain), which is domestically available, and boostered at 5 weeks after the first vaccination. Sera were collected at the same intervals from peripheral bloods and hog cholera virus-specific antibodies were detected by an indirect immunofluorescent assay (IFA) [17]. For the IFA test, PK-15 cell monolayers infected with hog cholera virus (LOM strain) were prepared in 96-well test plates. The cell suspension (0.2 ml, 1×10^5 cell/ml) was transferred to each well of 96-well plates and incubated for 24 h at 37 °C. The monolayers were washed 3 times with phosphate buffered saline (PBS pH 7.4) and 0.2 ml of the virus solution ($10^{3.0}$ $TCID_{50}$/ml) was transferred to each well. Virus-infected plates were incubated for 72 h at 37°C. After the incubation for 72 h, the medium in the plates was replaced by 5% cold acetone in absolute ethanol (0.1 ml/well). The plates were stored at 20°C until use. Negative and positive control sera were included in each test. IgG IFA test using commercial anti-swine IgG fluorescein isothiocyanate conjugate (FITC) was performed as previously described [16].

Statistical analysis

The Student's *t* test was used to compare the mean values between two groups. One way analysis was performed with the mean values from the pigs of T-1 or those from pigs of T-2 versus those from pigs of T-3. Also, one way comparison was done for three treatments versus those of control. Data were expressed as the mean ± SE.

Results and Discussion

Proportional comparison of porcine leukocyte subpopulations in pigs treated with non-specific immuno-

Table 1. Proportional comparison of porcine leukocyte subpopulations in pigs treated with non-specific immunomodulators

	Weeks post-application		
Group	0	5	8
<MHC class II cells			
T-1	14.65±2.07	13.33±2.15[a]	19.58±1.21[a,b]
T-2	10.20±0.40[a,b]	11.19±1.38[a,b]	12.73±2.28[a,b]
T-3	15.55±2.75	16.30±2.17	26.80±3.81
Con	14.57±3.18	20.59±1.77	23.56±2.47
<$CD2^+$ cells>			
T-1	76.25±1.29	63.03±5.58[a]	69.86±4.45[a]
T-2	68.85±1.15[a]	63.84±5.62[a]	66.90±4.15[a,b]
T-3	74.18±5.71	69.34±2.00[a]	77.69±3.00
Con	81.10±2.84	75.90±3.21	80.96±1.72
<$CD4^+$ cells>			
T-1	23.48±4.22[a,b]	18.64±3.26[a]	29.58±1.71
T-2	36.80±0.80[a,b]	23.70±2.78	26.72±2.92[b]
T-3	30.68±2.65	24.03±3.19	31.80±1.82
Con	32.97±0.94	26.03±3.55	29.31±3.04
<$CD8^+$cells>			
T-1	35.77±4.52	37.45±6.26[a]	42.23±4.62[a]
T-2	24.35±0.65[a,b]	42.80±2.85[a,b]	36.62±1.98[a,b]
T-3	36.80±4.78	37.11±1.38[a]	51.10±5.32
Con	37.12±2.75	50.88±4.07	57.48±4.97
<B cells>			
T-1	7.98±1.67[b]	3.94±0.60[a,b]	16.10±0.90
T-2	9.55±0.15[b]	6.73±0.78[a]	11.63±1.39[a,b]
T-3	11.78±0.75	8.77±1.21	18.43±2.83
Con	10.45±2.23	13.37±4.28	18.33±1.29
<N cells>			
T-1	16.18±1.77	21.84±2.77	23.40±2.00
T-2	18.70±0.70	21.89±3.10	19.76±1.57[a]
T-3	20.35±2.64	26.21±1.74	23.83±4.04
Con	16.93±3.00	22.91±2.60	25.19±1.23

All pigs were vaccinated with 1 ml of attenuated live hog cholera virus vaccine intramuscularly at 28-32 days old and boostered at 63-68 days old.

T-1; pigs treated with a basic diet sprayed with PowerFeel™ solution to be 0.05%(w/w) in a final concentration

T-2; pigs treated with a basic diet mixed with SuperFeed™ to be 3%(w/w) in a final concentration

T-3; pigs treated with a diluted PowerFeel™ solution(1:500, v/v) as drinking water

Con; pigs supplied with a basic diet and tap water

[a]; significant difference against that of control($p<0.05$)

[b]; significant difference against that of T-3($p<0.05$)

modulators was summarized in Table 1. There was a significant decrease in proportions of subpopulation expressing MHC-class II in T-1 and T-2 pigs at 5 and 8-weeks PA, compared to those of T-3 pigs treated with a diluted IAMC solution (1 : 500) as drinking water or non-treated control pigs ($p<0.05$). There was no significant difference in MHC class II between T-3 and control pigs. However, significant changes were observed in the proportions of T lymphocyte ($CD2^+$) of the treated groups versus those of control group during the experimental period. Those expressing $CD4^+$ showed a significant decrease in T-1 versus control at 5-weeks PA ($p<0.05$), and in T-2 versus T-3 at 8-weeks PA. In addition, those expressing $CD8^+$ showed significantly lower mean values at 5- and 8-weeks PA, whereas the change was also significant in T-2, compared to T-3 ($p<0.05$). The proportions of surface IgM^+ B lymphocytes were decreased with significant changes at 5-weeks PA and in T-2 at 8-weeks PA. In addition, no significant chang was observed in the proportion of N cells, but that of N lymphocytes was distinct for that of T-2, compared to that of control at 8-weeks PA.

The enhancement of host defense system using non-specific immunomodulators could be elucidated by monoclonal antibodies specific to leukocyte differentiation molecules of animal species [3]. In this study, the IMAC-treated groups showed a significant decrease in the proportions of subpopulations expressing MHC-class II, $CD2^+$, $CD4^+$, $CD8^+$ and surface IgM^+ B lymphocytes at 5-weeks after the first vaccination of modified live hog cholera virus. In addition, significant decreases were observed in the proportions of MHC-class II, $CD2^+$ and $CD8^+$ lymphocytes at 3-weeks after booster injection. The results of this study proved previous reports that a modified live hog cholera virus (LOM strain) vaccine had the pathogenicity like other virulent strains of hog cholera virus, but that the virulence of the virus is much less than that of them [6, 13]. Along with severe disease of leukocyte and lymphocyte counts, each number of MHC class II, $CD1^+$, $CD2^+$, $CD4^+$, $CD8^+$, $CD4^+CD8^+$ and surface IgM^+ B cells was decreased severely two days after inoculating virulent ALD strain of HCV, and each count of subpopulations was not recovered during the experiment period until death of pigs [6, 12]. In pigs vaccinated with modified live hog cholera virus, absolute numbers of leukocyte, lymphocyte and lymphocyte subpopulations except for the null cells were decreased transiently from 2 to 8 days after inoculation [5]. In addition, IAMC-treated pigs showed significant reductions of the lymphocyte subpopulations compared with the control, suggesting that the virus replication and persistence in the leukocytes after hog cholera virus infection might be altered, thereby resulting in the most important outcomes in the pathogenesis of hog cholera in pigs. Therefore, the same

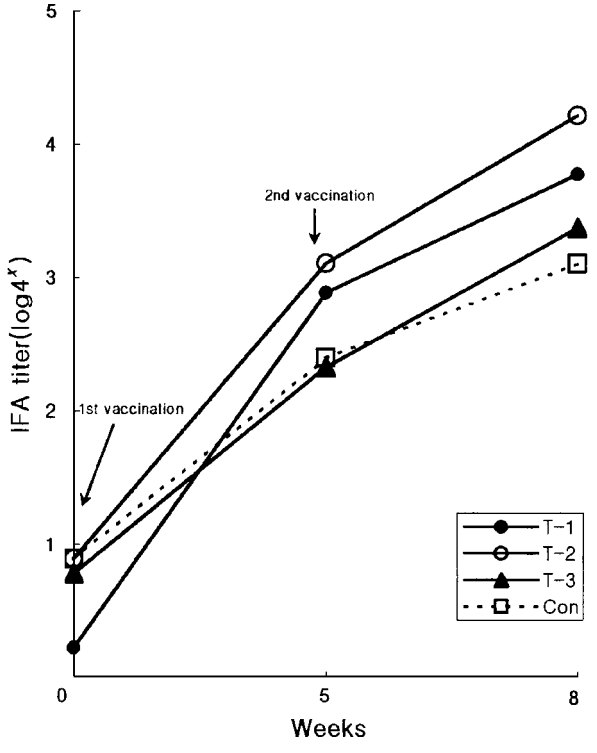

Fig 1. Mean IFA titers of attenuated live hog cholera virus vaccine in pigs treated with an ionized alkali mineral complex (Refer to the footnotes of Table 1)

pathogenicity of modified live hog cholera virus in pigs treated with the IAMC should be discussed whether this pathogenicity is closely related with the mechanism of production of antibody or not.

The vaccination effect of modified live hog cholera virus (HCV) was proved through the detection of HCV-specific antibodies. Mean antibody titers of each group against HCV were dramatically increased after booster injection (Fig. 1). The humoral immune responses of T-1 and T-2 were greater than those of T-3 or control group.

According to the maternal antibody derived from sows, it may have potentials to interfere with specific viral replication after vaccination with a live virus [7]. In this experiment, variable maternal antibody titers against hog cholera virus, when vaccinated, might influence the proliferation of hog cholera vaccine virus. However, maternal antibody titers of 1:16~1:32 against hog cholera virus would not reduce the efficacy of modified live hog cholera virus (LOM strain) vaccine [7]. Also, the titers measured by indirect immunofluorescent antibody test may not correlate directly with virus neutralizing ability. In addition, the marked correlation between the titer of neutralizing antibodies and the protective effects of modified live virus hog cholera vaccine approves that humoral immune mechanisms are important host defence reactions in hog cholera virus infection [7]. But cell-

mediated immunity also plays an important role in hog cholera virus infection.

The humoral immune responses of T-1 and T-2 were greater than those of T-3 or control group. A report supports the result of our study, suggesting that in pigs the Ig-containing cells isotypes of the various systemic lymphoid organs together did not correlate with the Ig-isotype concentration in serum [1]. Several studies indicate that cell-mediated immunity is not a critical factor but humoral immunity plays a major role in protection against hog cholera virus infection [2, 14]. The infection of lymphocytes may, therefore, contribute to the depletion in their numbers after infection and lead to defective antibody production during virulent classical swine fever virus infection [9]. On the contrary, even though there were severe reductions of specific lymphocyte subpopulations in pigs treated with the IAMC in this study, the establishment of solid immunity remains to be elucidated in the future whether it may be due to the same mechanism as pigs recovered from the natural infection of the virus used to obtain higher antibody titer or not.

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