Changes of Immunoglobulins and Lymphocyte Subpopulations in Peripheral Blood from Holstein Calves Challenged with *Escherichia coli* Lipopolysaccharide

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ABSTRACT : The objective of this study was to characterize serum immunoglobulins and lymphocytes subpopulations in the peripheral blood mononuclear cells (PBMCs) of Holstein calves in response to lipopolysaccharide (LPS) challenge from *Escherichia coli*. Fourteen calves received subcutaneous injections of *E. coli* LPS at 10 weeks of age, and six calves were injected with saline as a control. The concentrations of total serum IgG and the relative amount of LPS-specific IgG in calves challenged with LPS were significantly higher ($p<0.05$) compared to control animals and LPS challenge significantly increased ($p<0.05$) the percentage of CD5$^+$ and CD21$^+$ T cells in PBMCs. Meanwhile, LPS challenge significantly increased ($p<0.05$, $p<0.01$) the percentage of CD8$^+$ and CD25$^+$ T cells in peripheral blood mononuclear cells (PBMC) at 7 and 14 Day-post LPS challenge (DPLC), respectively. The composition of CD4$^+$CD25$^+$ T cells and CD8$^+$CD25$^+$ T cells from calves challenged with LPS was also higher ($p<0.05$ and $p = 0.562$, respectively) than those of control calves at 14 DPLC. In conclusion, LPS challenge not only induces production of IgG with expression of B-cell immune response related cell surface molecules, but also stimulates activation of T-lymphocytes in PBMC. Our results suggest that LPS challenge in calves is a good model to elucidate cellular immune response against Gram-negative bacterial infections. (**Key Words :** Calf, T-lymphocytes, Immunoglobulins, Lipopolysaccharide)

INTRODUCTION

Calves have heightened susceptibility to a variety of infectious diseases due to the developmental immaturity of their immune system combined with the effects of high stress conditions, including weaning and transport (Kim et al., 2009). Morbidity and mortality in calves are commonly associated with bacterial infections (Rings, 1985), especially *Escherichia coli* (Acres, 1985). Although calf physiological responses to lipopolysaccharides (LPS) have been described previously (Kinsbergen et al., 1994; Elsasser et al., 1996; Deluyker et al., 2004), no study has yet assessed the responses of serum immunoglobulins and T-cell subpopulations to LPS challenge in calves. During Gram-negative bacterial infections, calves are exposed to

and exhibit increased amounts of LPS, which may lead to serious cardio-respiratory, hematological, metabolic and endocrine changes evoked by cytokines (Kinsbergen et al., 1994). LPS is a pyrogen that is shed from bacterial surfaces during replication or death (Burvenich et al., 2003). LPS evokes a broad spectrum of functional responses from both lymphoid and non-lymphoid cells, including thymus-independent (TI) antigen-specific responses and non-specific polyclonal activation of B cells to secrete immunoglobulins. LPS is also a strong stimulatory molecule for antigen presenting cells (APC) such as macrophages and dendritic cells via toll-like receptor-4, resulting in the release of various cytokines (e.g., IL-6, TNF-α and IL-12). These cytokines then induce adaptive immune responses, including the activation of T cells. Previous studies have suggested that some T-lymphocytes respond to stimulation with LPS (David et al., 1997; Goodman and Weigle, 1979; Vogel et al., 1983). T-lymphocytes can be subdivided into αβ T-lymphocytes, which include CD4$^+$ (T-helper) lymphocytes and CD8+ (T-cytotoxic or T-suppressor) lymphocytes, and γδ

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T-lymphocytes (Sordillo et al., 1997). The α-chain of the IL-2 receptor, CD25, is expressed on activated immune cells. The high-affinity IL-2 receptor allows T-cell proliferation and differentiation (Foote et al., 2005). The lymph node homing receptor, L-selectin (CD62L), is required for entry of naïve lymphocytes into lymph nodes through high endothelial venules. Adequate expression is necessary for a successful innate immune response against bacterial infection (Foot et al., 2005). We investigated the production of immunoglobulins, CD5, and CD21 expression in peripheral blood mononuclear cells to characterize B-cell immune responses. In addition, we investigated modifications of T-lymphocyte populations, particularly αβ T-lymphocytes, through the expression of characteristic antigens (CD4 and CD8) on their cell surface following LPS challenge. We also analyzed the activation states of T-lymphocytes through the co-expression of activation markers (IL-2 receptor) and adhesion molecules (L-selectin). The hypothesis of the current study was that subcutaneous LPS injection would not only induce production of immunoglobulins in B-cells, but also stimulate the activation of T-cell subpopulations. The objective of the present study was to examine changes of circulatory lymphocyte populations and immunoglobulins in response to LPS challenge in Holstein calves.

MATERIALS AND METHODS

Animals and experimental procedures

All experimental procedures were reviewed and approved by the Institutional Review Board on the Use of Animals in Research of the National Livestock Research Institute, South Korea. Twenty Holstein calves (49.45±0.52 kg of body weight and 42±2 d of age) were used in this study. At 10 weeks of age, 14 calves were subcutaneously injected with *E. coli* lipopolysaccharide (LPS) (Sigma-Aldrich, St. Louis, MO, USA) 500 ng/kg of body weight. An additional six calves were injected with the same amount of saline and used as a control. LPS was reconstituted with non-pyrogenic phosphate-buffered saline (PBS) (Gibco, Invitrogen, Cergy Potoise, France). Blood samples (8 ml) were collected for flow cytometry analysis in evacuated tubes with EDTA (BD Vacutainer, BD-Plymouth, UK) at -2, 7, 14, 21, and 28 days-post LPS challenge (DPLC). Additional blood samples (5 ml) were collected into evacuated tubes with anti-coagulant lithium-heparin at the same time to determine total and LPS-specific Igs. Plasma was harvested from anti-coagulated blood after centrifugation at 1,600×*g* at 4°C for 20 min and stored at -80°C until subsequent assays were conducted.

ELISA assay for serum immunoglobulins

The concentrations of immunoglobulins (IgG, IgA, and IgM) in plasma were determined using commercial ELISA kits (Bethyl Laboratory, Montgomery, TX, USA). In brief, 96-well immunoplates (Nalgene Nunc International, Rochester, NY, USA) were coated with the appropriate bovine Ig capture antibody and incubated for 1 h. The plates were then washed three times with washing buffer (0.05% Tween 20 in PBS) and blocked with blocking buffer (0.05% Tween 20 in PBS) for 30 min. After washing, the plates were incubated with bovine plasma and respective standard proteins for 1 h at room temperature (RT). The detection antibody conjugated with biotin was added to the plates after washing and incubated for 1 h at RT. Specific binding was detected using streptavidin-horseradish peroxidase (HRP) and tetramethybezidine (TMB) substrate (Sigma-Aldrich, St. Louis, MO, USA). The reaction was stopped with 2 N H_2SO_4. Absorbance was measured at 450 nm using a microplate reader (Molecular Devices, Sunnyvale, CA, USA). LPS-specific Igs (IgG, IgA, and IgM) in the serum samples were determined by indirect ELISA. Relative amounts of LPS-specific Igs were measured using a bovine Ig ELISA assay kit (Bethyl Laboratories) according to the manufacturer's recommendations. In brief, the wells of 96-well immunoplates (Nalgene Nunc International) were coated with LPS solution (1.4 μg/ml, LPS dissolved in PBS) and coating buffer (0.05 M carbonate-bicarbonate, pH 9.6) and incubated overnight at 4°C. The plates were then washed three times with washing buffer (0.05% Tween 20 in PBS) and blocked with washing buffer (0.05% Tween 20 in PBS) for 2 h. After washing, the plates were incubated with diluted bovine serum for 2 h at room temperature. Anti-bovine IgG, IgA or IgM HRP-conjugated antibody was added to the plates after washing, and incubated for 2 h at RT. Specific binding was detected using streptavidin-HRP and TMB substrate (Sigma-Aldrich). The reaction was stopped with 2 N H_2SO_4. The absorbance was measured at 450 nm using a microplate reader (Molecular Devices).

Immunophenotyping of PBMC

Blood was diluted four-fold with PBS. Peripheral blood mononuclear cells (PBMC) were separated from buffy coat using Ficoll-Paque^TM plus (GE Healthcare, Piscataway, NJ, USA) density gradient centrifuged at 1,300×*g* for 20 min at 18°C with no break. The PBMC were washed twice with PBS and were used to analyze the compositions of lymphocytes in PBMC. Monoclonal antibodies (mAbs) were purchased from VMRD, Inc. (Pullman, WA, USA). Isotype-matched phyerythrin (PE)-labeled rat anti-mouse IgM and fluoresceinisothiocyanate (FITC)-labeled rat anti-mouse IgG_1 (Becton Dickinson, San Jose, CA, USA) were used as secondary mAbs (Table 1). Flow cytometry was used to determine the proportions of CD4^{+}, CD5^{+}, CD8^{+}, CD21^{+}, CD25^{+} lymphocytes and co-expressing CD25 and

Table 1. Primary and secondary antibodies used in immunostaining of bovine peripheral blood mononuclear cell (PBMC)

Antigen	Clone	Ig isotype	Main cellular expression
CD4	GC550A1	IgM	T subset (helper/inducer)
CD5	B29A	IgG_{2a}	B-1 cell and T cells
CD8	BAQ111A	IgM	T subset (cytotoxic/suppressor)
CD21	GB25A	IgG_1	B cells
CD25	CACT116A	IgG_1	IL-2 receptor (activated T cells)
CD62L	Du1-29	IgG_1	L-selectin (cell migration)

CD62L in $CD4^+$ and $CD8^+$ T cells in PBMC. 1×10^6 cells of PBMC suspended in 0.1 ml PBS were incubated for 20 min at 4°C with 1 μl of mAbs to CD4, CD5, CD8, CD21, CD25 and/or CD62L. The cells were washed three times with PBS and then were incubated for 20 min at 4°C with 1 μl of appropriate mAbs to PE-labeled rat anti-mouse IgM, FITC-labeled rat anti-mouse IgG_1 or FITC-labeled rat anti-mouse IgG_{2a}. Then, the cells were washed three times with PBS, suspended in 0.2 ml PBS and examined for fluorescence using a FACScanto flow cytometer (BD Bioscience). In each experiment, the cells incubated with isotype matched antibody, unrelated control antibody to use as negative controls. All data acquired was analyzed using CellQuest software (Becton Dickinson).

Statistical analysis

Data from flow cytometry and serum immunoglobulins were analyzed by ANOVA using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC, USA). When a significant effect of time or LPS challenge was found, differences among means were tested using the least significant difference (LSD) procedure of SAS (1996). Effects were considered significant at $p<0.05$.

RESULTS

Effect of LPS challenge on immunoglobulins in peripheral blood

To determine the effects of LPS challenge on production of immunoglobulins, we measured concentrations of total serum and LPS specific Igs. LPS challenge significantly induced production of serum IgG in the circulation. As shown in Figure 1A, calves who received LPS challenges (n = 14) showed a significant increase in total plasma IgG at 21 and 28 DPLC compared to those of control calves (n = 6) (mean±SE; 30.0±4.3 vs. 18.3±2.1 and 23.7±4.1 mg/ml vs. 14.7±0.9 mg/ml). However, such change was not observed in total serum IgA and IgM (Figure 1). The changes in relative amounts of LPS-specific immunoglobulins are presented in Figure 2. Calves challenged with LPS showed an increase of LPS-specific IgG, while control calves did not show such elevation (Figure 2A). LPS-specific IgG concentrations in calves with LPS challenge were significantly higher ($p<0.05$) at 14, 21, and 28 DPLC than those of saline injected animals. LPS challenge did not influence the concentration of LPS-specific IgA and IgM (Figure 2B and C).

Effect of LPS challenge on lymphocyte composition of PBMC

In order to investigate whether LPS challenge modulates B cell immune response related surface molecules expression in PBMC with immunoglobulin production, we observed the composition of $CD5^+$ and $CD21^+$ T lymphocytes in PBMC by single color flow cytometric analysis. A significant increasing of CD5 and CD21 expression in PBMC was observed. Percentage of CD5 in calves challenged with LPS was significantly ($p<0.05$) higher control animal at 5 DPLC (78.41±4.2% vs. 64.23±6.1%) and expression CD21 in PBMC from calves challenged with LPS was significantly ($p<0.05$) increased at 5 and 14 DPLC (Figure 3A and B). However, the percentage of $CD5^+CD21^+$ cells in calves with LPS challenge was not different in the experimental period (Figure 3C). To determine the effects of LPS challenge on sub-population of T cells in PBMC, we observed the composition of $CD4^+$, $CD8^+$, and $CD25^+$ T lymphocytes in PBMC. The profiles clearly show a significant increase of CD8 and CD25 expression in PBMC from calves challenged with LPS compared to those from control calves at 7 and 14 DPLC, respectively (13.3±1.5% vs. 9.7±1.2%; 10.2±1.2% vs. 5.3±0.3%) (Figure 4B and C). On the contrary, LPS challenge did not influence the percentage of $CD4^+$ T cells in PBMC (Figure 4A). Although the percentage of $CD4^+$ T cells increased in calves challenged with LPS at 7 DPLC, there was no difference between these calves and those challenged with saline. The calves with LPS challenge showed a significantly lower ($p<0.05$, $p<0.01$) ratio of CD4:CD25 and CD8:CD25 than saline injected calves at 14 DPLC (2.25±0.2% vs. 1.1±0.1%; 1.5±0.1% vs. 0.8±0.3%, respectively). LPS challenge did not influence the ratio of CD4:CD8 in PBMC (Figure 5A).

Effect of LPS challenge on CD25 and CD62L expression in T cell subsets

CD25 and CD62L expression on $CD4^+$ and $CD8^+$ T lymphocytes was measured by double color flow cytometric
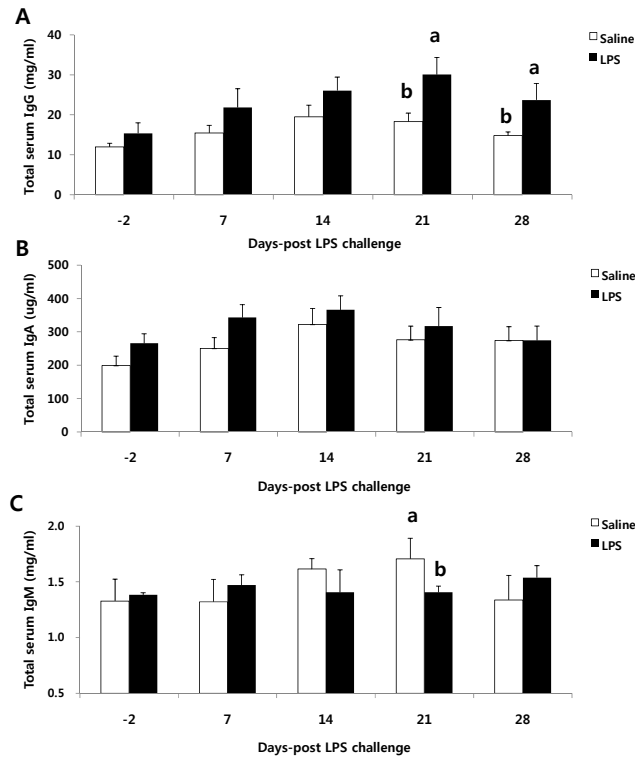
Figure 1. Profiles of total IgG (A), IgA (B), and IgM levels (mean±SE) from calves challenged with saline or LPS. Value represents mean±SE. Saline indicates a group of animals challenged with saline and LPS indicates a group of animals challenged with LPS; DPLC = days post-LPS challenge (0 DPLC = day of LPS challenge). [ab] Means with different letters differ significantly between groups (p< 0.05).
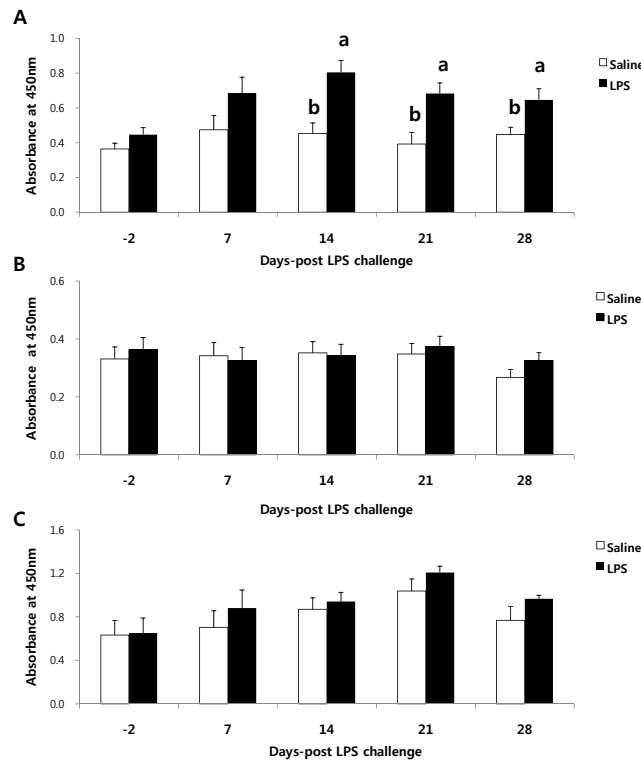

Figure 2. Profiles of relative amounts of LPS-specific IgG (A), IgA (B), and IgM (C) from calves challenged with saline or LPS. Value represents mean±SE. DPLC = days post-LPS challenge (0 DPLC = day of LPS challenge). [ab] Means with different letters differ significantly between groups (p<0.05).
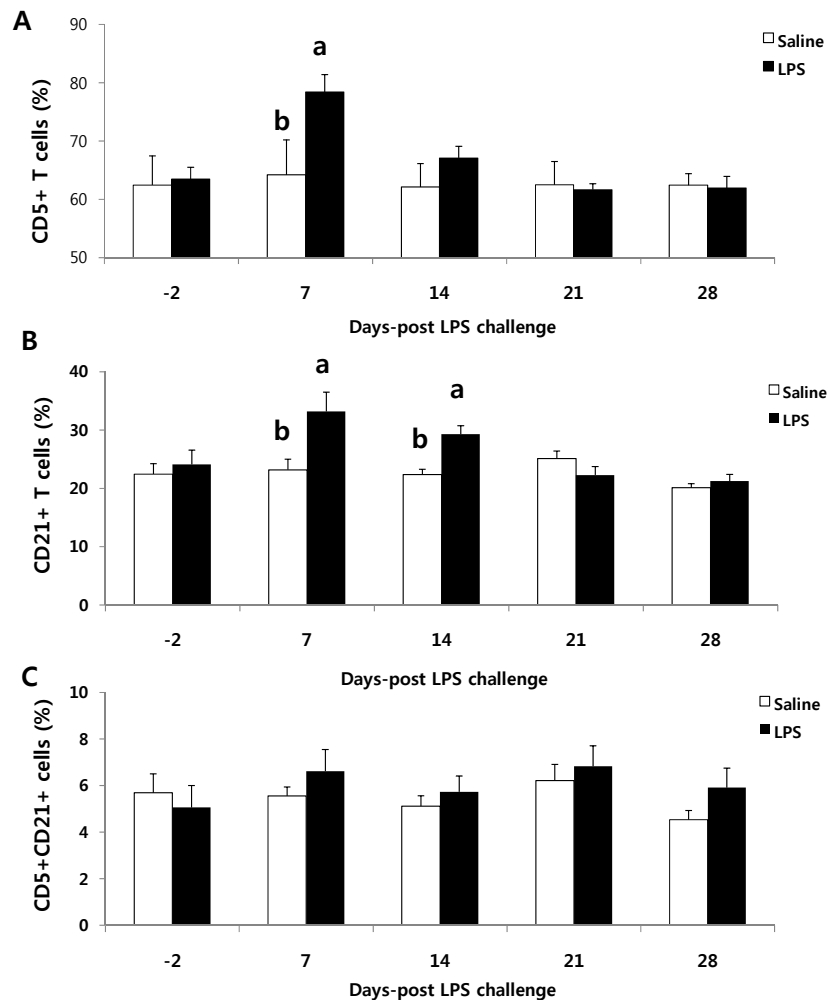
Figure 3. Influence of LPS challenge on the composition of lymphocytes from PBMC in calves. Calves were challenged with LPS or saline and blood was drawn at indicated time points. PBMC were separated by Ficoll gradient, labeled with antibodies, and analyzed by using flow cytometry. Percentages of all cells, staining with the indicated markers are depicted. Value represents mean±SE. Change of percentages (%) of $CD5^+$ (A) $CD21^+$ (B), and $CD5^+CD21^+$ (C) cell subsets in PBMC from calves. DPLC = days post-LPS challenge (0 DPLC = day of LPS challenge). [ab] Means with different letters differ significantly between groups ($p<0.05$).
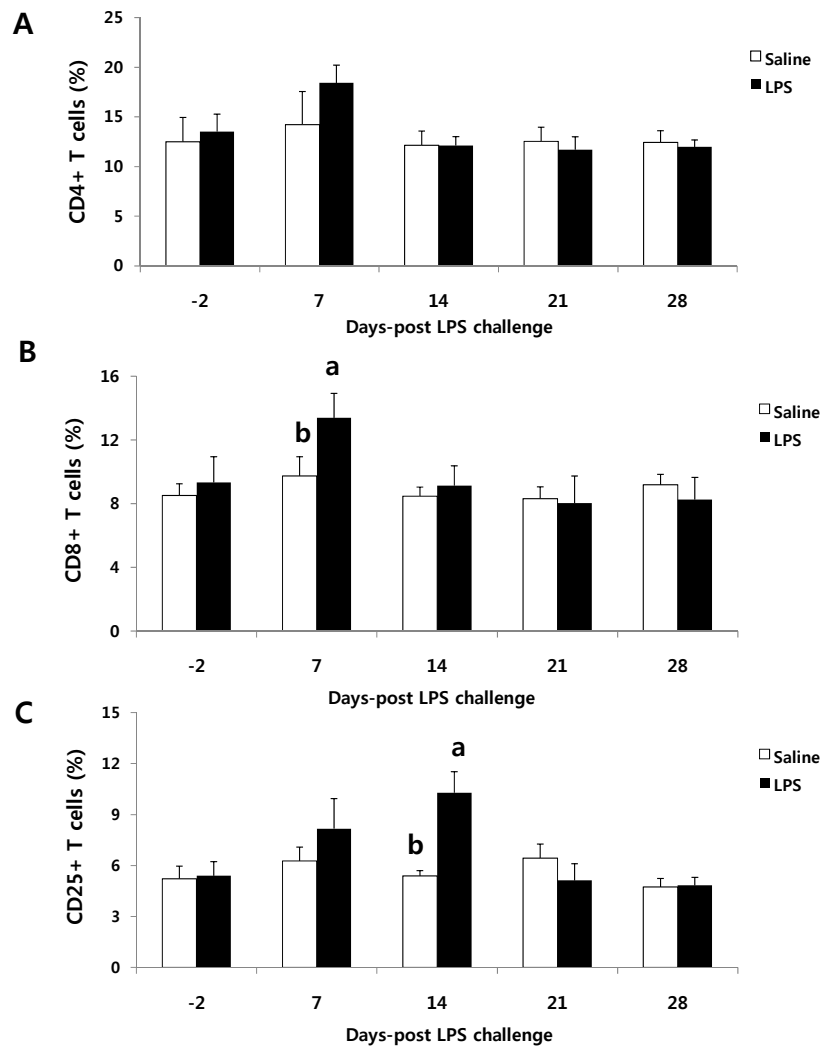
Figure 4. Influence of LPS challenge on the composition of lymphocytes from PBMC in calves. Calves were challenged with LPS or saline and blood was drawn at indicated time points. PBMC were separated by Ficoll gradient, labeled with antibodies, and analyzed by using flow cytometry. Percentages of all cells, staining with the indicated markers are depicted. Value represents mean±SE. Change of percentages (%) of CD4^{+} (A) CD8^{+} (B), and CD25^{+} (C) T cell subsets in PBMC from calves. DPLC = days post-LPS challenge (0 DPLC = day of LPS challenge). [ab] Means with different letters differ significantly between groups (p<0.05).
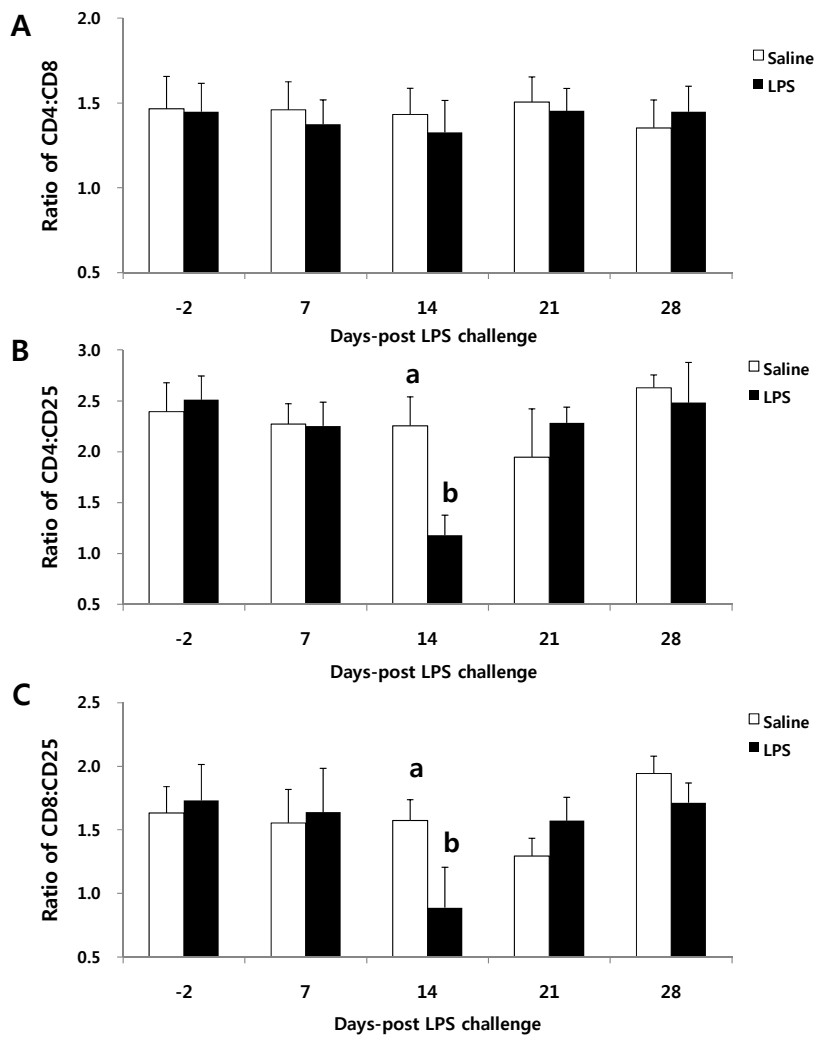
Figure 5. The ratio of lymphocytes from PBMC in calves challenged with LPS. Calves were challenged with LPS or saline and blood was drawn at indicated time points. PBMC were separated by Ficoll gradient, labeled with antibodies, and analyzed by using flow cytometry. Value represents mean±SE of determinations from all animals per group/sampling time. The ratio of CD4:CD8 (A), CD4:CD25 (B), and CD8:CD25 (C). DPLC = days post-LPS challenge (0 DPLC = day of LPS challenge). [ab] Means with different letters differ significantly between groups (p<0.05).
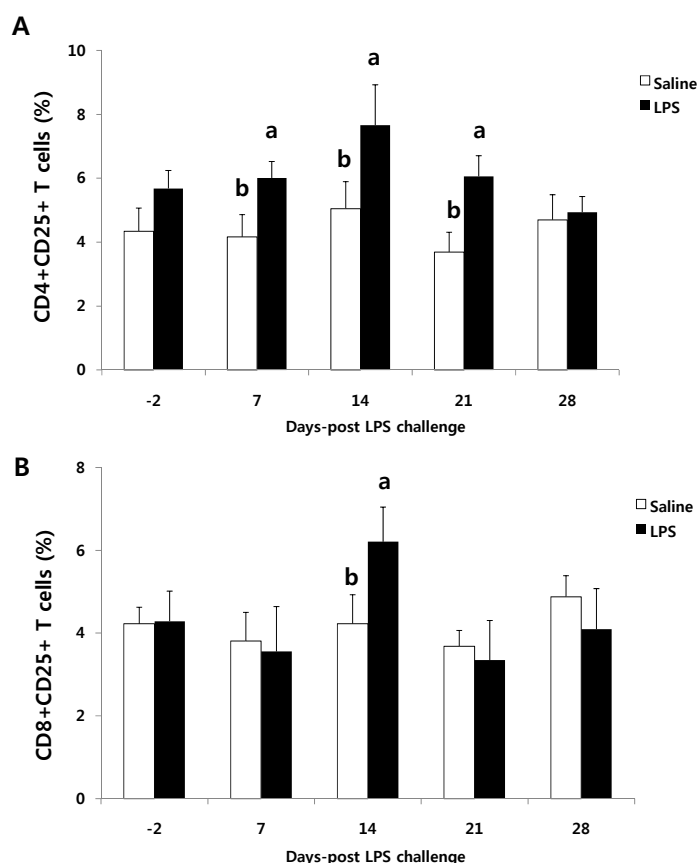
Figure 6. Changes on the activation state (CD25 expression) of lymphocytes from PBMC in calves. Calves were challenged with LPS or saline and blood was drawn at indicated time points. PBMC were separated by Ficoll gradient, labeled with antibodies, and analyzed by using flow cytometry. Percentages of all cells, staining with the indicated markers are depicted. Value represents mean±SE. Change of percentages (%) of $CD4^+CD25^+$ (A) and $CD8^+CD25^+$ (B) T cell subsets in PBMC from calves. DPLC = days post-LPS challenge (0 DPLC = day of LPS challenge). [ab] Means with different letters differ significantly between groups (p<0.05).

analysis. The profiles clearly show an increase of CD25 expression in PBMC from calves after LPS challenge. The percentage of $CD4^+CD25^+$ T-cells in calves with LPS challenge was significantly higher (p<0.05) than in control calves at 7, 14, and 21 DPLC (6.0±0.5% vs. 4.1±0.6%; 7.6 ±1.2% vs. 5.0±0.8%; 6.0±0.6% vs. 3.6±0.6%, respectively) (Figure 6A). Calves with LPS challenge also showed a significantly higher (p<0.05) percentage of $CD8^+CD25^+$ T cells than saline injected animals at 14 DPLC (6.2±0.8 vs. 4.2±0.7) (Figure 6B). There was no significant change in CD62L expression on T cells in calves challenged with LPS compared to the control group (Figure 7).

DISCUSSION

LPS is a major component of the outer membrane of Gram-negative bacteria and is an ideal molecule for recognition of bacteria by the immune system. Consequently, the LPS of different species have comparable activities regardless of the pathogenic properties of the corresponding bacteria. Purified LPS caused a number of

pathophysiological reactions in experimental animals that were strikingly similar to those seen during infections with Gram-negative bacteria (Marina et al., 2001). LPS not only induces numerous biologic effects, including mitogenic and polyclonal B cell response (Andersson et al., 1972), but is also a potent stimulant for macrophages and results in their activation (Morrison et al., 1979). In the present study, 14 calves were challenged with LPS from *E. coli* by subcutaneous injection at 10 weeks of age in order to characterize the profiles of LPS-specific immunoglobulins (Igs) and composition of lymphocytes subpopulation in the peripheral blood mononuclear cells (PBMC). Our results showed that LPS challenge induced effective B-cell immune responses in which the total serum IgG concentration increased. Importantly, the amount of LPS-specific IgG was significantly increased when compared to those of control animals. However, LPS injection did not influence total or LPS-specific IgA and elevation of IgM levels was not observed in this experimental period. It might be expected that more sampling time points between 7 to 14 DPLC are necessary to detect the elevation of IgM
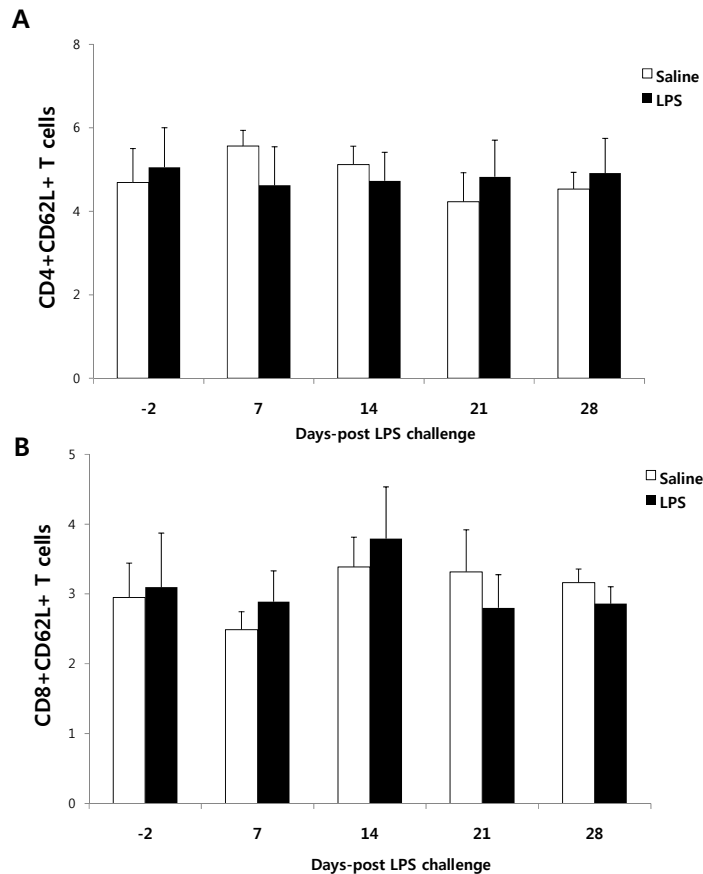
A

B

Figure 7. Expression of migratory molecule (L-selectin) on T-lymphocytes from PBMC in calves challenged with LPS or saline and blood was drawn at indicated time points. PBMC were separated by Ficoll gradient, labeled with antibodies, and analyzed by using flow cytometry. Percentages of all cells, staining with the indicated markers are depicted. Value represents mean±SE. Change of percentages of CD4$^+$CD62L$^+$ (A) and CD8$^+$CD62L$^+$ (B) T cell subsets in PBMC from calves. DPLC = days post-LPS challenge (0 DPLC = day of LPS challenge). [ab] Means with different letters differ significantly between groups (p<0.05).

before IgG production. It is to note that LPS challenge modulated B-cell activation related cell surface molecules expression in PBMC. In the present study, cell surface molecule CD5 and CD21 was increased in PBMC after LPS challenge. It is known that CD5$^+$ B cell is the predominant B cell population in neonatal period and thought to produce antibodies and evidence suggests that the CD5 molecule may play a role in cell signaling (Jyonouchi et al., 1990; Foote et al., 2007). Expression of CD21 forms a complex with CD19 and CD81 to form the B cell coreceptor and it can serve as antigen-presenting cells, secrete cytokines, and differentiate into plasma cells that produce and secrete immunoglobulins (Foote et al., 2007). These results suggested that LPS induced immunoglobulins production might be generated with B-cell response cell surface molecule expression such as CD5 and CD21.

On the other hand, it has been demonstrated in mice that LPS-induced polyclonal activation is not affected by the presence or absence of T cells because polyclonal activation of B cells to secrete Igs is dependent only upon direct interaction between LPS and its target B cell (Coutinho et

al., 1976). Although LPS-induced proliferation of lymphoid cells is thought to be primarily restricted to B cells, a number of reports suggest potential effects on T cells. Several previous studies demonstrated that T cells not only influence LPS-induced polyclonal Ig production, but can also be stimulated directly by LPS (Shinohara and Kern, 1976; Vogel et al., 1983). For example, David et al. (1997) reported that LPS not only causes polyclonal activation of B cells and stimulation of antigen-presenting cells but also stimulates both CD4$^+$ and CD8$^+$ T cells under *in vivo* conditions. On the other hand, specific immunity, particularly regarding lymphocyte populations, in immune response against bacterial infection is a major area of research (Riollet et al., 2001).

In the present experimental model, LPS injection significantly increased the percentage of CD8$^+$ (suppressor or cytotoxic) T cells and CD25$^+$ (activation marker, IL-2 receptor α) T cells in PBMC from calves with LPS challenge at 7 DPLC and 14 DPLC, respectively. Although the percentage of CD4$^+$ T cells was slightly increased in calves challenged with LPS at 7 DPLC, there was no

significant difference (p>0.05) between saline-injected and LPS-injected calves. LPS injection induced changes in the CD4:CD25 and CD8:CD25 ratios. A larger percentage of $CD25^+$ lymphocytes compared with $CD4^+$ and $CD8^+$ lymphocytes (the number of $CD25^+$ lymphocytes was multiplied by 1.90 between LPS-injected and control calves, compared with 1.29 and 1.37 for $CD4^+$ and $CD8^+$ lymphocytes, respectively) lead to a decreased ratio of CD4:CD25 and CD8:CD25. These results present that LPS challenge influenced the composition of T- lymphocytes, indicating elevated percentage of $CD8^+$ and $CD25^+$ lymphocytes in PBMC. In the present study, we investigated the co-expression of CD25 and CD62L (adhesion molecule, L-selectin) of bovine $CD4^+$ and $CD8^+$ T cells in response to LPS challenge in order to compare the activation statuses of other cell surface markers. CD25, the alpha-chain of the IL-2 receptor, is expressed on activated T-cells and B-cells. Activated T cells enable a fully functional receptor that can bind to IL-2 with high affinity, which in turn activates the proliferation of T cells. Activated T-helper cells are essential in determining antibody class switching, in the activation and growth of cytotoxic T cells, and in maximizing bactericidal activity of phagocytes such as macrophages. In cattle, proliferating $CD4^+$ and $CD8^+$ cells exhibit increased expression of CD25 (Waters et al., 2003). In addition, expression of CD25 on $CD4^+$ and $CD8^+$ cells has been used to monitor the response of bovine T-cells to various bacterial infections (Sandbulte and Roth, 2002; Vanden Bush and Rosenbusch, 2003; Nonnecke et al., 2005). In the present study, LPS challenge increased the percentage of $CD4^+CD25^+$ (activated T-helper cells) and $CD8^+CD25^+$ T cells (activated cytotoxic T cells). These results indicate that LPS challenge induces activation or proliferation of $\alpha\beta$ T-lymphocytes in PBMC. It is also possible that LPS challenge induced the secretion of cytokines such as TNF-α, IL-1, IL-12, and IFN-γ as an innate immune response, and that these cytokines stimulated the T-lymphocytes in the circulation. Further studies of cytokine production in bovine immune cells are necessary to elucidate the mechanism of T cell stimulation in calves challenged with LPS. On the other hand, adhesion molecule L-selectin (CD62L) facilitates adherence of leukocytes to vascular endothelium and subsequent migration of leukocytes between endothelial cells into infected tissue (Perkins et al., 2001). Its expression on murine lymphocytes is downregulated after polyclonal and antigenic stimulation *in vivo* and *in vitro* (Dailey, 1998). In *Mycobacterium bovis*-infected cattle, CD62L expression is decreased in antigen-activated $CD4^+$ and $CD8^+$ cells (Waters et al., 2003). However, in the present study, LPS challenge did not influence the co-expression of cell migration molecules on $CD4^+$ (helper T cells with adhesion molecule, CD62L) and $CD8^+$ cells (cytotoxic T cells with adhesion molecule, CD62L). These results suggest that LPS

challenge may not be the primary cause for the migration of T cells.

In conclusion, we found that LPS from *E. coli* induced polyclonal activation to produce immunoglobulins, particularly LPS-specific IgG in the circulation with CD5 and CD21 expression. Furthermore, LPS challenge also induced changes in the composition of T-lymphocytes in PBMC. Results presented in this study indicate that LPS challenge not only increased the composition of $CD8^+$ T-lymphocytes and $CD25^+$ cells (activated cells) in PBMC but also induced co-expression of CD25, an activation molecule, in $CD4^+$ and $CD8^+$ lymphocytes.

In the present model, LPS challenge induced antibody production with CD5 and CD21 expression in the circulation of experimental animals. Specifically, calves challenged with LPS showed elevated expression of $CD8^+$ and $CD25^+$ lymphocytes as well as co-expression of $CD25^+$ in $CD4^+$ and $CD8^+$ lymphocytes in PBMC. These results suggest that T cells can be stimulated *in vivo* by LPS injection. Further studies of changes in cytokines and lymphocyte population after LPS challenge are necessary in order to elucidate the mechanism of action of LPS in the context of immune response in calves. Our results suggest that the present experimental approach is useful to elucidate the cellular immune response in Gram-negative bacteria infections in calves.

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