Journal of Animal and Veterinary Advances 11 (24): 4603-4610, 2012
ISSN: 1680-5593

Cytokine and Chemokine Microarray Profiles in Lung and Hilar Nodes from Pigs after Experimental Infection with Actinobacillus Pleuropneumoniae

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Abstract: The objective of this study was to determine cytokine and chemokine microarray profiles in lung and Hilar Nodes (HN) from pigs infected with Actinobacillus Pleuropneumoniae (APP). Twenty pigs were randomly assigned to one of two groups: Control Group (CG) and inoculated with APP (TG). The infected-APP pigs' lung exhibited significantly ($p<0.05$) greater levels of chemokines CCL2, CCL20, IL8 and slightly increase levels of chemokines CCL4, CCL5 and CXCL2 while significantly($p<0.05$) decrease levels of chemokines CXCL10 and CXCL12. APP infection significantly ($p<0.05$ or 0.01) stimulated expression of cytokines IL-18, IL-6, TNF, GM-CSF, CASP3, CASP8 and significantly ($p< 0.05$ or 0.01) suppressed expression of cytokines CD40, IRF1 in lung. Cytokines in infected-APP pigs' lung, IL-1A, IL-27, IRF3, IL-10 were slightly increased and CASP1, IRF7, IL-12B, IL-2 were slightly decreased. Relative cytokine and chemokine microarray data in HN indicated that APP infection significantly ($p<0.05$) stimulated expression of cytokine IL-6 and significantly ($p<0.05$ or 0.01) suppressed expression of cytokines CXCL12, CD40 and CASP1. In conclusion, 26 cytokine and chemokines mRNA expression levels in lung and HN obtained from infected-APP or control swines were elucidated in this study. This research provided evidence that the increased severity of lesions in the infected-APP swines was associated mainly with alterations of cytokine and chemokines microarray profiles, especialy in lung. The changes of all the cytokines in lung and HN can lead stem cells to produce granulocytes (neutrophils, eosinophils and basophils) and monocytes and also promoted neutrophil and macrophages to phagocytose bacterial and foreign antigen at the site of inflammation. Defense function of pig infection with APP was enhanced while immune function was weakened.

Key words: Cytokine, chemokine, Actinobacillus pleuropneumoniae, microarray, China

INTRODUCTION

Porcine *Actinobacillus pleuropneumonia* is a highly contagious, fibrinous, hemorrhagic and necrotizing pneumonia with high mortality or localized lung lesions in chronically infected pigs (Shope, 1964). Actinobacillus Pleuropneumoniae (APP) is the causative agent of porcine pleuropneumonia a disease occurring worldwide and causing significant economic losses in the swine industry world wide (Aarestrup and Jensen, 1999; Bosse *et al*., 2002; Gutierrez-Martin *et al*., 2006; Matter *et al*., 2007; Rycroft and Garside, 2000). Pleuropneumonia results from the uncontrolled release of pro-inflammatory mediators and cytokines in response to APP or its product Lipopolysaccharides (LPS) (Udeze *et al*., 1987; Baarsch *et al*., 1995; Choi *et al*., 1999). The LPS excreted by APP appears to be associated with the early inflammatory response (Udeze *et al*., 1987; Baarsch *et al*., 1995; Bertram, 1985; Idris *et al*., 1993). Overproduction of macrophage-derived mediators such as oxygen radicals, nitric oxide, prostaglandins and pro-inflammatory cytokines such as Tumour Necrosis Factor-a (TNF-α) and Interleukin (IL)-1 has been shown to be responsible for the inflammatory reactions (Baarsch *et al*., 1995; Bertram, 1988; Xing *et al*., 1994; Morrison *et al*., 2000). Likewise, bioactive protein and or mRNA coding for IL-10, IL12p35, TNF-α and IFN-α have been shown to be up-regulated after infection with APP *in vivo* or *in vitro* (Baarsch *et al*., 2000; Huang *et al*., 1999; Wattrang *et al*., 1998; Cho and Chae, 2002; Cho *et al*., 2005). These studies have focused on a few selected genes using techniques such as quantitative Real-Time reverse-transcriptase Polymerase Chain Reaction (qRT-PCR), Northern blotting or *in situ* hybridization.

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Using cDNA microarrays, Moser *et al.* (2004) identified 307 anonymous transcripts in blood leukocytes obtained from pigs that were severely affected by experimental infection with APP; Hedegaard *et al.* (2007) found three subsets of genes that were consistently expressed at different levels depending upon the infection status.

In this study, researchers investigated the gene expression profiles of APP-infected lung and Hilar Node (HN) from swines by Agilent Whole Porcine Genome Oligo (4X44K) Microarrays. Swines were experimentally inoculated with APP and microarray analyses were conducted on lung tissues and HN from challenged versus non-challenged pigs. The 26 cytokine and chemokines mRNA expression levels in lung and HN obtained from infected-APP by microarray data or control pigs were elucidated in this study. Further investigation of the roles of these the locally-produced proinflammatory cytokines in the lung lesion and HN will greatly enhance the understanding of the pathogenesis of APP infection.

MATERIALS AND METHODS

Animals and bacterial inoculation: All animal procedures were performed according to protocols approved by the Biological Studies Animal Care and Use Committee of Sichuan province, P.R. China. Twenty, 12 weeks old male castrated Danish Landrace/Yorkshire/Duroc crossbred swines from a healthy APP free herd were divided equally into a Control Group (CG) and a Treatment Group (TG), isolation rearing, respectively. Swines from the TG (pigs 1-10) were inoculated with 1 mL containing 3.8×10^{7} cfu mL^{-1} APP serotype I (provided by the Animal Biotechnology Center, Laboratory of Animal Disease and Human Health, Sichuan Agricultural University) by atomizing inhalation into each nostril. Ten swines from the CG (pigs 11-20) were inoculated with physiological saline (0.9% wt./vol. NaCl) by the same means.

Blood sample collection: Swines were blood sampled via precava venipuncture on post-inoculated 48 h. For this procedure, the swines were led gently to a holding pen with a squeeze chute facility and were blood sampled with minimal restraint. Blood samples were collected into 1×6 mL Ethylenediamine Tetraacetic Acid (EDTA) tripotassium tubes (Jiangsu Kangjian Medical Apparatus, China) for haematological analysis.

Haematology: Unclotted whole tripotassium EDTA blood samples were analysed using an Abacus Junjor Vet haematology analyser (Diatron, GmbH, Wien, Austria) equipped with software for pig blood. White Blood Count (WBC), totals of Lymphocytes (LYM), Granulocytes (GRA) and Monocytes (MON), Red Blood Cell count (RBC), contents of Haemoglobin (HGB), Mean Corpuscular Haemoglobin Concentration (MCHC), Percentage of Haematocrit (HCT) and total of Platelet (PLT) were measured. The N:L ratio was also calculated.

Tissue sample collection: In the CG (pigs 11, 12 and 13) lung tissue and HN were collected from three pigs after abattage and used for total RNA extraction and pathological analysis. Another three pigs from the TG (pigs 1, 2 and 3) were sacrificed 48 h post-inoculation and their HN collected.

Microarray hybridizations and data analysis: Total RNA was extracted from tissues using Trizol reagent (Invitrogen). RNA was purified and DNase treated using the RNeasy QIAGEN RNeasy® Mini kit (QIAGEN). The cDNA was synthesized from 2 μg of total-RNA using the direct cDNA Labeling System. Aminoallyl-cRNA was synthesized from cDNA using the Superscript Indirect cDNA Labeling System (Agilent). The cRNA was purified and DNase treated using RNeasy QIAGEN RNeasy® Mini kit. RNA integrity was confirmed using a bioanalyzer (model 2100; Agilent Technologies, Palo Alto, CA) according to the manufacturer's protocol. Labeling and hybridization of the cRNA was performed with Agilent Whole Porcine Genome Oligo (4X44K) Microarrays (one-color platform) at the National Engineering Center for Biochip at Shanghai, according to the manufacturer's protocols. The slides were scanned and analyzed using the Histogram Method with default settings in an Agilent G2565AA and Agilent G2565BA Microarray Scanner System using SureScan Technology. Microarrays data were obtained by data processing and normalization.

Statistical analysis: Statistical analysis was performed with SPSS 11.5 for Windows.

RESULTS AND DISCUSSION

1PP model of artificial infection APP were established by using the Aerosol Method: The symptoms and lung lesions observed in TG swines were typical of infection with APP. Swines developed hyperthermia (40.6-42.0°C), dyspnea and anorexia 24-48 h post-inoculation (p.i.) with APP. Two swines with respiratory distress died 36-48 h p.i. At autopsy, the lungs were severely affected by acute multifocal fibrinonecrotizing and hemorrhagic pneumonia complicated by acute diffuse fibrinous pleuritis. The HN were enlarged and congested. No lesions in CG were observed. The infected swines had lung and pleural

lesions of variable severity consistent with acute pleuropneumonia whereas the surrounding lung and pleural tissue appeared normal.

Histopathological lesions were not observed in the lung tissues and HN of the CG swines. However, lesions were apparent in the lung and HN of the TG swines. These histopathologic changes were characterized by hemorrhage, neutrophils, macrophages and lymphocytes infiltration, fibrinous exudation vascular thrombosis, necrotic focus and lung edema. The histopathologic changes in HN were characterized by loose medulla, congestion, edema, fibrinous exudation and neutrophils infiltration.

Influence of APP infection on the blood index of swines: Compared with those of CG, the numbers of White Blood Count (WBC) and the totals and percentages of Granulocytes (GRA), Monocytes (MON) were all inreased ($p<0.01$) while Lymphocytes (LYM) were dereased ($p<0.01$) in the peripheral blood of swines in TG. The results showed that the blood defensive functions of infected-APP swines were enhanced while the blood immunity functions were weakened. Compared with those of CG, the N:L ratio of TG increased ($p<0.01$) . There was a significant effect on the N:L ratio of infected-APP swines (Table 1).

Relative cytokine and chemokine mRNA expression data: Relative cytokine and chemokine mRNA expression data (Table 2) indicated that TG swines HN had significantly ($p<0.05$) greater stimulation of cytokines IL-6 while CXCL12, CD40, CASP1 were significantly ($p<0.05$ or 0.01) suppression. The infected-APP swines lung exhibited significantly ($p<0.05$) greater levels of chemokines CCL2, CCL20, IL8 and slightly upregulation levels of chemokines CCL4, CCL5 and CXCL2 while significantly ($p<0.05$) downregulation levels of chemokine CXCL10 and CXCL12. Compared with those of CG, the relative gene expression of many proinflammatory cytokines IL-18, IL-6, TNF, GM-CSF, CASP3, CASP8 in TG swines lung exhibited significantlygreater levels ($p<0.05$ or 0.01) and with no activation of cytokines CASP10, ICAM-1, ICAM-2 or IL-5 while the cytokines CD40, IRF1 levels were significantly downregulated ($p<0.05$ or 0.01). Cytokines IL-1A, IL-27, IRF3, IL-10 were slightly increased in infected-APP swines whilst IFN-DELTA-1, CASP1, IRF7, IL-12B, IL-2 were slightly decreased.

Leukocytes are involved in defending the body against both infectious disease and foreign materials. GRA, MON and LYM are the main types of leukocytes among the GRA including neutrophils, eosinophils and basophil. The concentration of neutrophil, MON, LYM

Table 1: The blood indicators of inoculated and non-inoculated APP swines

Items	CG	TG
WBC ($\times10^3$ cells μL^{-1})	12.89±1.2000^{A}	21.57±2.300^{B}
GRA ($\times10^3$ cells μL^{-1})	3.50±0.7600^{A}	17.08±1.990^{B}
GRA (%)	27.06±5.1000^{A}	79.23±4.560^{B}
LYM ($\times10^3$ cells μL^{-1})	9.35±1.1900^{B}	4.14±1.030^{A}
LYM (%)	72.30±0.5100^{B}	19.10±4.030^{A}
MON ($\times10^3$ cells μL^{-1})	0.08±0.0100^{A}	0.36±0.120^{B}
MON (%)	0.60±0.0900^{A}	2.14±0.810^{B}
G:L(Ratio)	0.38±0.0900^{A}	4.36±1.170^{B}
RBC ($\times10^6$ cells μL^{-1})	6.56±0.4300	6.74±0.560
HGB (g dL^{-1})	11.59±9.5400	11.08±5.260
MCH (pg)	17.68±1.4600	16.48±1.160
MCHC (g dL^{-1})	34.04±1.1700	33.40±0.780
HCT(L L^{-1})	33.96±2.1200	33.08±1.750
MCV (fL)	51.88±3.3600	49.25±2.310
RDWcv	24.16±4.4600	26.05±3.320
RDW sd	48.54±6.5100	49.20±3.860
MPV (fL)	14.68±3.4600	12.68±2.010
PCT (L L^{-1})	2.05±1.2300	1.21±0.620
PLT ($\times10^3$ cells μL^{-1})	1296.13±566.37	912.00±349.0
PDWcv	42.63±2.6000	40.90±2.960
PDWsd	21.05±5.5600	17.75±4.090

Values within a column followed by different capital letters were significantly different ($p<0.01$) between two groups. Values within a column followed by different small letters were different ($0.01<p<0.05$) between two groups. Values within a column followed by same letters were not different ($p>0.05$)

Table 2: Effect of APP infection on the relative gene expression of cytokines and chemokines in swines' lung and HN

	Relative gene expression[a]			
	Lung		HN	
Cytokine and chemokine	CG	TG	CG	TG
CCL2	14.35±0.21^{A}	17.01±0.39^{Cb}	15.89±0.40^{BCa}	15.68±0.67^{B}
CCL20	8.92±0.88^{Aa}	11.59±1.73^{b}	13.47±0.45^{Bbc}	14.05±1.29^{Bc}
CCL21	9.97±0.54^{A}	9.90±0.77^{A}	13.89±0.59^{B}	12.94±0.97^{B}
CCL4	11.60±0.47	13.04±2.11	11.59±0.19	11.93±0.32
CCL5	8.64±0.64	10.03±2.22	8.92±0.62	8.23±0.13
CXCL10	11.29±0.89^{Ab}	10.12±0.20^{Aa}	13.41±0.11^{B}	12.79±0.07^{B}
CXCL12	13.48±0.10^{Ab}	13.16±0.03^{Aa}	17.14±0.20^{C}	15.91±0.13^{B}
CXCL2	13.92±0.07^{ABab}	15.90±1.36^{Bb}	11.82±0.51^{A}	13.33±2.10^{a}
CD14	10.32±0.12^{Aa}	12.86±0.71^{B}	11.91±0.39^{b}	12.37±1.23^{B}
CD40	14.51±0.19^{B}	13.97±0.04^{A}	16.26±0.17^{D}	15.56±0.24^{C}
GM-CSF	9.98±0.29^{B}	11.89±0.19^{C}	7.85±0.28^{A}	7.71±0.67^{A}
IL-1A	9.48±0.28^{A}	12.40±1.17^{B}	8.29±0.23^{Aa}	9.84±0.57^{Ab}
IL-6	8.24±0.01^{Aa}	11.59±0.87^{B}	10.10±0.11^{b}	11.92±1.39^{Bc}
IL-8	10.94±0.03^{a}	15.30±2.55^{Bb}	9.22±1.14^{A}	11.41±2.94^{a}
IL-12B	6.23±0.43^{ABa}	5.32±0.55^{A}	9.91±0.24^{Cc}	8.20±1.31^{BCb}
IRF1	14.16±0.52^{b}	13.42±0.25^{Aa}	15.09±0.12^{Bc}	14.41±0.41^{B}
TNF	8.03±0.03^{Aa}	8.81±0.55^{b}	9.12±0.16^{B}	9.22±0.29^{B}
IL-5	5.62±0.04^{Aa}	5.58±0.13^{Aa}	6.52±0.47^{B}	6.19±0.30^{b}
IL-10	11.60±0.64^{A}	12.45±0.68^{a}	13.67±0.43^{B}	13.91±1.11^{Bb}
IL-2	5.70±0.14^{ABa}	4.91±0.42^{A}	7.77±0.44	7.54±1.31^{BCb}
CASP1	13.10±0.44^{B}	12.00±0.06^{ABab}	12.49±0.79^{b}	11.54±0.10^{Aa}
CASP3	10.44±0.16^{a}	11.27±0.66^{b}	10.47±0.32^{ab}	11.15±0.14^{b}
CASP8	9.92±0.19^{Aa}	10.25±0.13^{Ab}	10.55±0.09^{B}	10.78±0.22^{B}
CASP10	15.64±0.17	16.09±0.38	15.08±0.60	16.23±1.02
ICAM-1	15.83±0.02^{B}	15.69±0.09^{B}	15.05±0.20^{A}	14.96±0.42^{A}
ICAM-2	12.99±0.24	12.47±0.28	12.49±0.33	12.77±0.43

[a]The relative gene expression levels are presented as the average ΔCt (after the Ct value for the housekeeping gene, ACTB was subtracted) and then averaged for each group. Values within a column followed by different capital letters were significantly different ($p<0.01$) between two groups. Values within a column followed by different small letters were different ($0.01<p<0.05$) between two groups. Values within a column followed by same letters were not different ($p>0.05$)

and G:L are associated with the defensive functions of the body. It has been reported that marked neutrophil and macrophage infiltration is an obvious feature of pulmonary lesions of acute APP infection (Bertram, 1988). MON exit the circulation and migrate into tissues whereupon they mature into macrophages. Thus, these cells play a part in the immune-inflammatory cascade by which activation of a small number of macrophages can rapidly lead to an increase in their numbers, a process crucial for fighting infection. In the present study, the totals of leukocytes and the totals and percentages of MON and GRA (including neutrophils, eosinophils and basophil) were all inreased while LYM were dereased in the peripheral blood of swines 48h p.i. with APP. The defensive functions of the infected-APP swines were enhanced by increasing the totals of GRA and MON while the infected-APP swines were at risk of infection other bacteria for decrease of the LYM totals. The reduction in LYM number may be attributed to the trafficking of LYM from general circulation into tissues and organs at risk of infection (Dhabhar, 2009). The changes of such a haematology were related to neutrophils and monocytes defending against bacterial infection and other very small inflammatory processe enhanced.

APP acutely induces marked infiltration of neutrophils followed with increased numbers of macrophages in the lungs (Bertram, 1988). The excessive infiltration of neutrophils and macrophages into the lung may play a destructive role (Strieter *et al*., 1992; Sibille and Reynolds, 1990). The mechanism of such an acute cellular infiltration after APP infection however is not fully clear. As chemoattractants, CCL2 (Carr *et al*., 1994; Xu *et al*., 1996), CCL4 (Bystry *et al*., 2001) and CXCL10 (Dufour *et al*., 2002; Angiolillo *et al*., 1995) can recruit MON from the blood to the sites of infection or tissue damage. In the present study, the levels of CCL2 mRNA in injury lung were upregulated >6 fold than those of non-injury lung while the levels of CXCL10 mRNA were significantly downregulated with CCL4 upregulation slightly. The main chemokines for MON from the blood to the injury lung tissues was CCL2 and CCL4, especially CCL2. The excessive infiltration of macrophages into the injury lung tissues may be mainly related to the upregulation of CCL2 mRNA. It has been recently shown that the mediators produced by the host rather than bacteria themselves may play a critical role in the excessive neutrophil infiltration (Huang *et al*., 1999). The protein chemoattractants include Il-8/neutrophil Activating Protein-I (NAP-1) (Strieter *et al*., 1992; Kunkel *et al*., 1991; Goodman *et al*., 1992) with IL-8 possibly playing a major role in neutrophil infiltration into the lung (Strieter *et al*., 1992). In this study, swine infected-APP lung tissues exhibited significantly greater levels of chemokine IL8 and >20 fold to those of no-infected swine. The excessive expression of IL-8 can not only promote neutrophils to phagocytose the antigen which triggers the antigen pattern toll-like receptors but also attract excessive neutrophils to the lung of inflammation. CCL20 and Chemokine (C-X-C motif) ligand 12 (CXCL12) are the chemokines strongly chemotactic for LYM (Hieshima *et al*., 1997). CCL20 was the major chemokines for recruiting lymphocytes in injury lung tissues and its level was upregulated >6 fold. CCL20 can also be induced by inflammatory cytokines such as TNF and IFN-γ and by microbial factors such as LPS (Schutyser *et al*., 2000). CXCL12 in infected-APP swine HN downregulated and CCL20 in infected-APP swine lung upregulated may be benefit of the lung tissues recruiting LYM from the HN and blood. Other chemoattractants as CCL5 recruiting for T cells, eosinophils and basophils and CXCL2 recruiting for polymorphonuclear leukocytes and hematopoietic stem cells (Wolpe *et al*., 1989; Iida and Grotendorst, 1990; Pelus and Fukuda, 2006) were both slightly increased in swine infected-APP lung tissues. The excessive infiltration of macrophages, neutrophils and lymphocytes into the lung in the infected-APP swines may be mainly related to the chemokines CCL2, IL8 and CCL20 mRNA significantly upregulation. Upregulations of chemokines as CCL2, IL8 and CCL20 caused a lot of effector cells as mainly monocytes, neutrophils and lymphocytes, recruiting in location of infection and caused location severe inflammation response.

Production of proinflammatory mediators in the lungs is an important feature of APP infection (Chen *et al*., 2011). Huang *et al*. (1999)'s study indicate that host inflammatory factors are involved in the pulmonary lesion development of APP infection. Several proinflammatory cytokines, particularly IL-1 and IL-8 in the lung are detected after APP inoculation (Baarsch *et al*., 1995). There were increased levels of TNF-α, IL-1 and IL-8 mRNA at the periphery of focal lung lesions after APP inoculation (Baarsch *et al*., 1995). Significant increases in IL-6 mRNA after infection with APP have previously also been observed in lung lavage as well as lung tissue using northern blotting and *in situ* hybridization (Baarsch *et al*., 1995; Myers *et al*., 2002). These proinflammatory mediators enhance inflammatory and immunological responses however, overproduction of such mediators in response to Gram-negative bacterial infection may induce pulmonary lesions, endotoxic shock and death (Tracey *et al*., 1986; Okusawas *et al*., 1988; Hack *et al*., 1997). In the present study, cytokines TNF-α, IL-1, IL-6, IL-8, GM-CSF and IL-18 mRNA were upregulated

significantly in the infected-APP swine' s lung tissues and IL-6 was also upregulated significantly in the infected-APP swine's HN. Of the cytokines a most important proinflammatory mediator, IL-18 plays multiple roles in chronic inflammation and in a number of infections and enhances both Th-1- and Th-2-mediated immune responses (Nakanishi *et al*., 2001). In the present study, IL-18 was activated and elevated over 5 fold in the infected-APP swine's lung. IL-18 is able to induce IFN-γ, GM-CSF, TNF-α and IL-1 in immunocompetent cells to activate killing by lymphocytes and to up-regulate the expression of certain chemokine receptors. IL-18 was possibly playing a major role in inducing severe inflammatory reactions in swine infected-APP lung. TNF-α can also promote inflammatory responses by inducing the production of other proinflammatory cytokines at the vicinity of the infection (Toews, 2001). As a potent chemoattractant for neutrophils and promotes the expression of adhesion molecules on endothelial cells, helping neutrophils migrate, TNF is elevated over 1.6 fold in the infected-APP swine lung. Acting as proinflammatory cytokine, IL-1α increases blood neutrophils and activates lymphocyte proliferation and induces fever; IL-6 is responsible for stimulating acute phase protein synthesis as well as the production of neutrophils in the bone marrow GM-CSF can stimulate stem cells to produce granulocytes (neutrophils, eosinophils and basophils) and monocytes. Activation of all these cytokines IL-1α, IL-6, GM-CSF can cause stem cells to produce effector cells (neutrophils, eosinophils, monocytes and basophils) and also induce neutrophils and macrophages to phagocytose bacterial and foreign antigens. The production of proinflammatory mediators TNF-α, IL-1, IL-6, IL-8, GM-CSF and IL-18 enhance inflammatory responses however, overproduction of such mediators in response to APP infection may be associated with the lung lesion development.

Acting as anti-inflammatory cytokine, IL-6 is mediated through its inhibitory effects on TNF and IL-1 and activation of IL-1ra and IL-10. In the present study, IL-10 mRNA levels were slightly increased in the infected-APP swine lung tissues and HN. IL-10 and IL-12 are most noted for their ability to regulate the balance between T Helper 1 (TH1) cells and TH2 cells (Moore *et al*., 1993; Trinchieri, 1995; Stern *et al*., 1996; Gately *et al*., 1998). TH1 cells secrete IL-12 and IFN-γ thus promoting cell-mediated immunity whereas TH2 cells produce IL-4, -5, -6, -10 and -13, thererby facilitating humoral immunity. IL-10 suppresses immune and inflammatory reactions by down-regulating the expression of Th1 cytokines (which can promote cell-mediated immunity such as IL-12, IL-2 and IFN-γ), MHC class II antigens and costimulatory molecules on macrophages and suppressing the antigen-presentation capacity of APC (Spits *et al*., 1992). CD40 is a costimulatory molecules found on APC and is essential in mediating a broad variety of immune and inflammatory responses including T cell-dependent immunoglobulin class switching, memory B cell development and germinal center formation (http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene and Cmd=Show Detail View and Term ToSearch=958). In the present study, the levels of CD40 were downregulated significantly. IL-12 is an essential inducer of Th1 cell development (Oppmann *et al*., 2000) and also necessary for the growth and function of T cells (Cantrell and Smith, 1984). IL-2 can facilitate production of immunoglobulins made by B cells and induce the differentiation and proliferation of NK cells (Waldmann and Tagaya, 1999; Waldmann, 2006). IL-5 is produced by T helper-2 cells and mast cells and functions are to stimulate B cell growth and increase immunoglobulin secretion. These cytokines (IL-2, IL-5 and IL12B, a subunit of IL12) mRNA levels were downregulated slightly in infected-APP swine lung and HN. The Interferon Regulatory Factor 1 (IRF1) level was significantly downregulated in infected-APP swines lung. IRF-1 plays roles in the immune response, regulating apoptosis, DNA damage and tumor suppression (http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene and Cmd = Show Detail View and Term To Search = 3659). The significance changes of cytokines led to a decrease of antigenic peptides which APC presented to T lymphocytes via the major histocompatibility complex and to alleviate immune response injury induced by infection at the site of inflammation, especialy in lung.

Caspase 3 and 8 were activated and both upregulated significantly in infected-APP swine lung while Caspase 1 involved in inflammasome formation and activation of inflammatory processes was slightly supressed. Caspases are a family of cysteine proteases that play essential roles in apoptosis, necrosis and inflammation (Alnemri *et al*., 1996). In the present study, infected-APP swines exhibited no activation of ICAM-1 and ICAM-2. When activated, leukocytes bind to endothelial cells via ICAM-1/LFA-1 and then transmigrate into tissues (Yang *et al*., 2005). In particular, ICAM-1 signaling seems to produce a recruitment of inflammatory immune cells such as macrophages and granulocytes (Etienne-Manneville *et al*., 1999). ICAM-2 mediates adhesive interactions important for NK-cell mediated clearance, lymphocyte recirculation and other cellular interactions important for immune response and surveillance (Bleul *et al*., 1996).

Induction of an innate immune response is important for the control and elimination of invading pathogens. In

conclusion, 26 cytokine and chemokines mRNA expression levels in lung and HN obtained from infected-APP or Control swines were elucidated in this study. APP infection was characterized by significantly increasing cytokines TNF-α, IL-1, IL-6, IL-18, GM-CSF, CASP3, CASP8 and chemokine CCL2, IL8 and CCL20, slightly increasing IRF3 and IL-10 mRNA and significantly decreasing cytokine CD40, IRF1 and chemokine CXCL10, CXCL12, slightly decreasing CASP1, IRF7, IL12B and IL2 mRNA, expression levels. The increase in cytokines and chemokines were correlated with increases in severity of microscopic lesions. Induced or repressed expression of the genes discussed above stimulated stem cells to produce granulocytes (neutrophils, eosinophils and basophils) and monocytes and promoted neutrophil and macrophages to phagocytose bacterial and foreign antigen at the site of inflammation.

CONCLUSION

This research provided evidence that the increased severity of lesions in APP infected swines was associated mainly with the alterations of cytokines and chemokines mRNA expression profiles.

ACKNOWLEDGEMENTS

This research was funded by the Natural Science Foundation of Science and Technology Department of Sichuan Province.

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