B- 3714
[1-5]

Indian J. Anim. Res.,
Print ISSN:0367-6722 / Online ISSN:0976-0555

AGRICULTURAL RESEARCH COMMUNICATION CENTRE
www.arccjournals.com/www.ijaronline.in

Cytokine response to killed *Staphylococcus pseudintermedius* antigen in dogs with skin diseases

Hridya Susan Varughese, M. Ananda Chitra*, Ranjani Rajasekaran, S.Rajalakshmi and G. Dhinakar Raj

Department of Veterinary Microbiology, Madras Veterinary College
Tamil Nadu Veterinary and Animal Sciences University, Vepery, Chennai-600 007, Tamil Nadu, India.
Received: 14-08-2018 Accepted: 27-11-2018 DOI: 10.18805/ijar.B-3714

ABSTRACT

The attempt was made to study the response of the dog having skin infection with various underlying causes to killed *S.pseudintermedius* antigen. PBMC were separated and sensitized with killed *S. pseudintermedius* for 24 hours. cDNA was made from extracted RNA and quantitative PCR were carried out for 8 cytokines with GAPDH as housekeeping gene. IL-6 was the cytokine which was expressed in a statistically significant high level in PBMC of healthy animals to killed antigen than PBMC of infected animals. Except for the IL-6, all other cytokines were expressed at high level in pyoderma animals than healthy control, demodicosis and allergy cases. In the present study, IL-1β, IL-8 and TNF-α were the cytokines that were up-regulated and, IL-6 and IFN-γ were down-regulated in demodicosis dogs with pyoderma at apparently significant level than the other tested cytokines. IL-4 was the only cytokine that was expressed in measurable quantity in allergic cases when compared to other cytokines. Thus, it was concluded that dogs with staphylococcal pyoderma infections developed a Th1/Th2 response to fight the infection.

Key words: Allergy, Cytokine, Demodicosis, Dog, Pyoderma, *Staphylococcus pseudintermedius*.

INTRODUCTION

Cytokines are soluble, hormone-like polypeptide mediators in inflammatory and immunoregulatory responses. Cytokines initiate their biological action by interacting with target cells bearing cytokine receptors. Once a cytokine binds to the target cell receptor, this can then trigger a cascade of cellular events that includes release of further cytokines, which may then stimulate release of other cytokines in other cells, possibly modulating release of initial cytokine (Sauder, 1990). Cytokines are a vital element of pathogenic mechanisms in skin diseases. Whether they are involved at all stages of the process, including initiation and propagation of the disease process, or whether they are primarily involved only in the propagation stages has yet to be determined.

In human and animals, skin infections are commonly caused by *Staphylococcus* species. Various components of Gram-positive microorganisms, such as cell wall constituents and exotoxins of *Staphylococcus aureus* (SA), have been shown *in vitro* to trigger different cascade systems and to induce production and release of cytokines. Many of these cytokines are believed to be important mediators of immune and inflammatory responses and to play a central role in orchestrating the pathogenesis of staphylococcal infections (van Deuren *et al*., 1992). Factors that contribute to the clinical outcome of SA include the virulence and antibiotic susceptibility of the infecting isolate (Takata *et al*., 2013), host innate and humoral immune responses (Santos *et al*., 2012) and the underlying condition of the patient (Fowler *et al*., 2005).

S. aureus molecules such as peptidoglycan and lipoteichoic acid (LTA) are potential stimulators of cytokine production (e.g. TNF-α, IL-1β, IL-6, IL-4, IL-8, IFN-γ and IL-12), in response to infection (Wang *et al*., 2000) but un-regulated cytokine production may contribute to *S. aureus* pathogenesis (McNicholas *et al.*2014). The most important pathogen that causes skin infection in dog is *Staphylococcus pseudintermedius* (SP). There is no study on the cytokine response of dogs to *S. pseudintermedius*. Hence, the attempt was made to study the response of the dog having skin infection with various underlying causes to killed *S. pseudintermedius* antigen.

MATERIALS AND METHODS

Ethical approval: No ethical approval was necessary for this study; however, we obtained informed consent from all the pet owners involved in this study for sample collection and we maintained the confidentiality of the diagnostic results.

Animals and study setting: Apparently healthy dogs brought for regular vaccination were served as control animals under Group I category. Dogs with skin infections brought to the Dermatology Unit of Teaching Veterinary Clinical Complex, Madras Veterinary College, Chennai during February 2018 to June 2018 were grouped into three

*Corresponding author’s e-mail: anandachitra.m@tanuvas.ac.in

study groups. Group II: Dogs with pyoderma but otherwise there was no underlying cause. Group III: Dogs with bacterial skin infections along with demodicosis. Group IV: Dogs with skin infections along with allergic conditions like food allergy/ flea allergy/ contact dermatitis. In each group, six animals were taken for this study. Whole blood of 5 ml was collected in EDTA vial from a total of 24 animals and skin swabs were also collected from the selected dogs so as to confirm the *Staphylococcus* infection. Isolation and identification of *S. pseudintermedius* isolates was done as described previously (Anandachitra *et al*., 2015).

Preparation of killed *Staphylococcus pseudintermedius* (SP) antigen: A killed SP suspension was prepared by suspending loopful of SP colonies grown on nutrient agar in 5 ml PBS and placing in 95°C water bath for 30 min. Suspension was pipetted thoroughly by drawing in and out, washed and diluted with PBS to match with McFarland standard # 3 and made aliquots and placed in -20° C for storage.

Separation of peripheral blood mononuclear cells (PBMC): PBMC was separated by density gradient centrifugation as per the method of Boyum (1968). The cells were suspended in RPMI 1640 (Gibco, Invitrogen, USA) media and viability of the cells was ascertained with trypan blue dye exclusion method.

Cell culture and stimulation: For the *in vitro* PBMC stimulation experiment, 1.5 x 10^6 cells were cultured in 6-well plate (Nunc, Thermo Fisher, USA). Culture medium contained 10% fetal bovine calf serum (HiMedia, India) with 1% penicillin and 1% streptomycin in RPMI 1640. The cells were stimulated with 10 µl of killed antigen containing 9 x 10^6 SP bacteria and the plates were incubated at 37° C for 24 hours at 5 % CO_2. Culture without killed antigen served as control in each category and cultures were done in triplicate.

RNA extraction and cDNA synthesis: Total RNA was extracted using Trizol (Invitrogen, Thermo Fisher, USA) and the final dried pellet was suspended in 20 µl of nuclease free water. The RNA concentration was quantified and was stored at –80°C. cDNA synthesis was performed as per manufacturer's instructions (BIO-RAD, U.S.A) in a 20 µl reaction.

Real time PCR assay: For qPCR assay, published primers of Tamura *et al*. (2014) were used and the list of the primers was given in Table 1. The immune response of dogs affected with various skin infections to killed SP was assessed by quantitative real time PCR employing eight cytokine genes namely IL-1β, IL-4, IL-6, IL-8, IL-10, IL-12, IFN-γ and TNF-α keeping the GAPDH gene expression as an internal control. The assay was carried out in a total volume of 10µL reaction mixture in Real time thermal cycler (LightCycler96, Roche, Switzerland). Each PCR reaction mix (10µL) consisted of 5µL of SYBR Green PCR Master mix (Takara, USA), 40 ng of cDNA and 5 pmol of primer. Amplification and detection of specific products were performed with the following cycle profile: 95°C for 10 min and 45 cycles PCR (95°C for 10 sec and 60°C for 10 sec and 72°C for 10 sec), followed by dissociation (95°C for 10 sec, 65°C for 60 sec and 97°C for 1 sec). The purity of the PCR products was monitored by analyzing the dissociation curves and by determining their size with agarose gel electrophoresis. The results were analyzed by delta delta cycle threshold method (ΔΔCt) of Pfaffl (2001) as per the formula:

$$\Delta\Delta Ct = \{(Ct\ target - Ct\ internal\ control)\ sample - (Ct\ target - Ct\ internal\ control)\ calibrator\}.$$

The change in the gene expression was calculated by 2 -ΔΔCt and the value indicated in an n-fold difference relative to the calibrator.

Table 1: Primers along with amplicon size, annealing temperature, and references used for amplification of canine cytokines.

Gene	Primer Sequence	Annealing tem. (°C)	Amplicon size (bp)	Ref.
GAPDH	F- ATCACTGCCACCCAGAAGAC R- TCAGCTCAGGGATGACCTTG	60	132	Tamura *et. al.,* 2014
IL-10	F- CGGGAGGGTGAAGACTTTCT R- GGCATCACCTCCTCCAAGTA	60	143	Tamura *et. al.,* 2014
IL-4	F- GGCATCACCTCCTCCAAGTA R- CGCTTGTGTTCTTTGGAGCA	60	143	Tamura *et. al.,* 2014
IFN-γ	F-GCGCAAGGCGATAAATGAAC R-CTGACTCCTTTTCCGCTTCC	60	81	Tamura *et. al.*, 2014
IL-1β	F- CAAGTCTCCCACCAGCTCTGTA R- GGGCTTCTTCAGCTTCTCCAA	60	80	Tamura *et. al.,*2014
IL-6	F-TTAAGTACATCCTCGGCAAAATCT R-CAGTGCCTCTTTGCTGTCTTCA	60	85	Tamura *et .al*., 2014
IL-8	F-CTCTCTGTGAAGCTGCAGTTCTG R-GGAAAGGTGTGGAGTGTGTTTTT	60	80	Tamura *et. al.,* 2014
IL-12p35	F-GTGCCTCAACCACTCCCAA R-CAATCTCTTCGGAAGTGCAGG	60	100	Tamura *et. al.,* 2014
TNF-α	F-TCTCGAACCCCAAGTGACAAG R-CAACCCATCTGACGGCACTA	60	153	Tamura *et. al.,* 2014

Statistical analysis: An oneway ANOVA test and Duncan Multiple Range Test (DMRT) were used to analyze the statistical significance of cytokine expression in different study groups of animals.

RESULTS AND DISCUSSIONS

Staphylococcus pseudintermedius was isolated and confirmed in two dogs in pyoderma group, one dog each in demodicosis and in allergy group. *Staphylococcus* bacteria were isolated from other animals but species level identification was not carried out. The level of expression of various cytokine in different study groups is given in Table 2. In this study, there was no statistical significant difference observed between healthy and infected groups for IL-4 and IL-12 cytokine expression ($p>0.05$). There is no available literature to support or contradict our findings in healthy dogs' *in vitro* cytokine response to SP antigen.

IL-6 was the cytokine which was expressed in a statistically significant ($p<0.01$) high level in healthy animals' PBMC to killed antigen than infected animals' PBMC. IL-8 was also expressed almost at equal level of IL-6 in this group. However, IFN-γ and IL-1β were expressed at very low level in healthy control group. In general, in healthy dogs, the serum cytokine level measured by multiplex bead immunoassay is reported to have IL-6 and IFN-γ below the level of detection (Richter *et al.*, 2018). The differential level of detection was reported for IL-8. Below the level of detection was reported by Bastien *et al.* (2015) and high level of detection was reported by von Pfeil *et al.* (2015). In healthy human beings, IL-1β, IL-2, IL-10 and IL-5 were reported to be below the level of detection. IL-6, IL-8, IL-12 and TNF-α were all in the quantifiable level. IFN-γ was detected at high level (Biancotto, 2013).

In this study, except for the IL-6 all other cytokines expressed at high level in pyoderma animals than healthy control, demodicosis and allergy cases. With the tested cytokines in this study, a high level of expression was found with IL-8 in pyoderma cases. IFN-γ was expressed more by at least 2.5 fold in pyoderma than other cases and it is statistically significant. There is no evidence to support our report in canine. High levels of IL-8 mRNA are present in psoriatic epidermis, especially in active psoriatic plaques (Camp *et al.*, 1989). IL-8, a potent neutrophil chemoattractant, is found to be increased in psoriasis along with IL-6 (Sauder, 1990). However, in the present study, IL-6 was relatively down regulated than IL-8 cytokine. This differential expression might be due to different host model (dog vs human) and type of sample (PBMC vs diseased skin).

Pyoderma gangrenosum (PG) patients showed over-expression of chemokines promoting neutrophil transend othelial migration into inflamed tissues, such as IL-8 than the healthy control (Marzano *et al.*, 2014). IL-1 promotes the production and release of both classic proinflammatory cytokines, such as TNF-α and IFN-γ, and a number of chemokines, notably IL-8 and RANTES (Dinarello, 2011).

Table 2: Mean and standard deviation values of different cytokine expression of PBMC of dogs with staphylococcal infection and various primary causes to killed *Staphylococcus pseudintermedius* antigen.

Condition	IFN-γ	IL-1β	IL-4	IL-6	IL-8	IL-10	IL-12	TNF-α
Healthy	0.055±0.027^{a}	0.220±0.187^{a}	1.420±0.176^{a}	15.087±4.748^{b}	15.827±4.708^{b}	3.096±1.194^{bc}	1.213±0.376^{a}	1.075±0.506^{ab}
Pyoderma	3.406±1.341^{b}	2.455±0.725^{b}	2.016±0.305^{a}	3.713±1.145^{a}	21.423±5.507^{b}	4.676±1.356^{c}	2.222±1.040^{a}	2.986±0.273^{c}
Demodicosis	0.399±0.153^{a}	2.549±1.606^{b}	1.440±0.440^{a}	0.049±0.004^{a}	5.134±2.184^{a}	1.685±0.600^{ab}	1.045±0.372^{a}	2.277±0.701^{bc}
Allergy	0.805±0.133^{a}	0.337±0.149^{a}	2.732±1.338^{a}	0.156±0.077^{a}	0.221±0.121^{a}	0.449±0.131^{a}	0.636±0.115^{a}	0.312±0.211^{a}
F value	4.414	6.93	0.727	23.171	13.717	6.532	1.906	5.064
P value	0.022 *	0.006**	0.587	0**	0.001**	0.007**	0.187	0.036*

*Significance at 5% level of significance, where $p \le 0.05$

**Significance at 1% level of significance, where $p \le 0.01$

Mean bearing same superscript does not differ significantly, whereas different superscript indicates significant difference at 5% or 1% level of significance.

In the dog, the association of demodicosis with pyoderma is well established and is probably the most severe consequence of demodicosis (Miller *et al.*, 2013). It is not known whether, as in humans, *Demodex* mites induce a proliferation of *Staphylococcus pseudintermedius*, or rather if this bacterium simply takes advantage of the epidermal barrier rupture that occurs in canine demodicosis (Ferrer *et al.*, 2014). In the present study, IL-1β, IL-8 and TNF-α were the cytokines that were up-regulated and, IL-6 and IFN-ã were down regulated in demodicosis dogs with pyoderma at apparently significant level than the other tested cytokines. There was no statistically significant level of production of IL-10 in demodicosis cases than the healthy cases. They were at least 2 fold higher expression of IL-1β in demodicosis cases than in allergy and healthy cases. It has been documented that once the demodicosis has developed in a dog, indicators of T-cell exhaustion, such as the low production of supportive/stimulatory cytokines (IL-2 and IL-21) and high levels of suppressive cytokines (IL-10 and transforming growth factor-β) along with low numbers of circulating CD4+ lymphocytes (Ferrer *et al.*, 2014). In contrast to our observation of slightly up-regulated TNF-α expression, the lower level of TNF-α mRNA was reported in dogs with both localized and generalized demodicosis than control dogs (Tani *et al.*, 2002). They have also reported that there was no significant difference in IFN-γ expression in dogs with localized demodicosis but in generalized demodicosis dogs, it was down-regulated at significant level. There were no significant different in IL-4 and IL-10 mRNA expression reported among the demodicosis and healthy control dogs in that study. The differential pattern of TNF-α expression between the studies might be due to difference in the study cases. In the present study, dogs suffering from demodicosis as well as pyoderma were taken but the other study was done in dogs with generalized and localized demodicosis and there was no report about the pyoderma condition. IL-10 levels in dogs with recurrent demodicosis were significantly higher than the healthy and first time infested dogs while no significant statistical difference was observed between healthy and first time infested dogs (Felix *et al.*, 2013). There was no difference in the ratio of CD4/CD8 ratio between control and demodicosis affected human being (Akilov and Mumcuoglu, 2004).

IL-4 was the only cytokines that was expressed in measurable quantity in allergic cases when compared to other cytokines in the present study. Gene and protein expression of IL-4 has been variably found in dogs with atopic dermatitis. In one study, IL-4 mRNA transcripts were found in the skin of both healthy and atopic dogs, albeit more frequently in atopic skin (Olivry *et al.*, 1999). Another study also found overexpression of IL-4 in atopic relative to healthy skin (Nuttal *et al.*, 2002). In contrast, others failed to detect IL-4 gene expression in either atopic or healthy dog skin, or any significant differences in mRNA expression between lesional, non-lesional or healthy skin (Schlotter *et al.*, 2011). Maina and co-workers (2017) reported that high IL-4 level was not changed even after the treatment in food allergic dogs whereas significant level of increase was observed in IFN-γ and IL-10 cytokines after treatment.

CONCLUSION

In conclusion, the present study reveals that in healthy animals, Th2 response cytokines (IL-6 and IL-10) were detected in their peripheral blood cells. However, in pyoderma affected dogs, there was an increased expression of IFN-γ, IL-1β, IL-8, IL-10 and TNF-α cytokines suggesting the presence of both Th1/Th2 responses for the resolution of the infectious process. In demodicosis with pyoderma dogs, increased pro-inflammatory cytokines (IL-1β and TNF-α) expression was observed. In contrast, in allergic dogs, there was no elevated Th cytokines noticed. In staphylococcal skin infected dogs, the data demonstrate that, no altered expression of IL-4 and IL-12 cytokines where as an increased expression of pro-inflammatory cytokines IFN-γ and TNF-α at $p<0.05$ level and IL-1β, IL-6, IL-8 and IL-10 cytokines at $p<0.01$ level was detected.

ACKNOWLEDGEMENT

The authors are thankful to DST-SERB, Government of India for funding this study (EMR/2016/006141) and Tamil Nadu Veterinary and Animal Sciences University Chennai, India for providing necessary infrastructure to carry out the research work.

REFERENCES

Akilov, O.E. and Mumcuoglu, K.Y. (2004). Immune response in demodicosis. European Academy of Dermatology and Venereology: *JEADV* :440–444 ,DOI: 10.1111/j.1468-3083.2004.00964.

AnandaChitra, M., Jayanthy, C. and Nagarajan, B. (2015). Detection andsequence analysis of accessory gene regulator genes of *Staphylococcus pseudintermedius* isolates, *Vet World*: **8**(7):902 -907.

Bastien, B.C., Patil, A. and Satyaraj, E. (2015). The impact of weight loss on circulating cytokines in Beagle dogs, *Vet Immunol Immunopathol*: **163**(3):174–182.

Biancotto, A., Wank, A., Perl, S., Cook, W., Olnes, M.J., Dagur, P.K., Fuchs, J.C., Langweiler, M., Wang, E. and McCoy, J.P. (2013). Baseline levels and temporal stability of 27 multiplexed serum cytokine concentrations in healthy subjects, *PLoS One:***8**(12): p.e76091

Böyum, A. (1968). Isolation of mononuclear cells and granulocytes from human blood. Isolation of monuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation at 1 g., *Scand J Clin Lab Invest Suppl:* **97**:77-89.

Camp, R.D.R., Fincham, N.J., Ross, J.S., Bacon, K.B. and Gearing, A.J.H.(1990). Leukocyte chemoattractant cytokines of the epidermis, *J of Invest Derm*: ***95***(6): 5108-5110.

Dinarello, C.A. (2011). A clinical perspective of IL-1â as the gatekeeper of inflammation. *Eur J Immunol*: **41**:1203–17.

Felix, A.O.C., Guiot, E.G., Stein, M., Felix, S.R., Silva, E.F. and Nobre, M.O. (2013). Comparison of systemic interleukin 10 concentrations in healthy dogs and those suffering from recurring and first time *Demodex canis* infestations, *Vet Parasit:***193**: 312– 315.

Ferrer, L., Ravera, I. and Silbermayr, K. (2014). Immunology and pathogenesis of canine demodicosis, *Vet. Dermatol.*:**25**: 427–465

Fowler, V.J., Justice, A., Moore, C., Benjamin, D.K. Jr,, Woods, C.W., Campbell, S., Reller, L.B., *et al* (2005). Risk factors for haematogenous complications of intravascular catheter-associated *Staphylococcus aureus* bacteremia, *Clin Infect Dis:***40**(5):695–703.

Maina, E., Devriendt, B. and Cox, E. (2017). Changes in cytokine profiles following treatment with food allergen-specific sublingual immunotherapy in dogs with adverse food reactions. *Vet Dermatol*: **28**: 612–e149.

Marzano, A.V., Fanoni, D., Antiga, E., Quaglino, P., Caproni, M., Crosti, C., Meroni, P. L. and. Cugno, M. (2014). Expression of cytokines, chemokines and other effector molecules intwo prototypic auto inflammatory skin diseases, pyodermagangrenosum and Sweet's syndrome. *Clin and Exp Immun.*:**178**: 48–56.

McNicholas, S., Talento, A.F., O'Gorman, J., Hannan, M.M., Lynch, M., Greene, C.M., Humphreys, H. and Fitzgerald-Hughes, D. (2014). Cytokine responses to Staphylococcus aureus bloodstream infection differ between patient cohorts that have different clinical courses of infection. BMC Infect Dis. **15**: (14)580.

Miller, W.H., Griffin, C.E. and Campbell, K.L. (2013**).** Parasitic diseases. In: *Mullerand Kirk's Small Animal Dermatology*, 7th edition. Philadelphia PA: W.B. Saunders Co.; 284–342.

Nuttall, T.J., Knight, P.A. and McAleese, S.M. (2002). Expression of Th1, Th2 and immunosuppressive cytokine gene transcripts in canine atopic dermatitis. *Clin Exp Allergy*; **32**: 789–795.

Olivry, T., Dean, G.A., Tompkins, M.B., Dow, J.L. and Moore, P.F. (1999). Toward a canine model of atopic dermatitis: amplification of cytokine gene transcripts in the skin of atopic dogs, *Exp derm* :***8*** ;204-211.

Pfaffl, M.W. (2001). A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. **29**(9):e45

Richter, K.R., Nasr1, A.N. and Mexas, AM. (2018). Cytokine concentrations measured by multiplex assays in canine peripheral blood samples, *Vet Path*: **55**(1) :53-67.

Santos, S.S., Brunialti, M.K., Rigato, O., Machado, F.R., Silva, E. and Salomao, R. (2012). Generation of nitric oxide and reactive oxygen species by neutrophils and monocytes from septic patients and association with outcomes, *Shock*: **38**(1):18–23.

Sauder, D.N. (1990). The role of epidermal cytokines in inflammatory skin diseases, *J Invest Dermatol.*:**95**: 27S-28S.

Schlotter, Y.M., Rutten, V.P., Riemers, F.M., Knol, E.F. and Willemse, T. (2011). Lesional skin in atopic dogs shows a mixed Type-1 and Type-2 immune responsiveness, *Vet immunol and immunopathol*: ***143***:20-26.

Takata, T., Miyazaki, M., Futo, M., Hara, S., Shiotsuka, S., Kamimura, H., Yoshimura, H., Matsunaga, A., *et al* (2013). Presence of both heterogeneous vancomycin-intermediate resistance and beta-lactam antibiotic-induced vancomycin resistance phenotypes is associated with the outcome in methicillin-resistant Staphylococcus aureus blood stream infection. *Scand J Infect Dis.*: **45**(3):203–212.

Tamura, Y., Ohta, H., Yokoyama, N., Lim, S.Y., Osuga, T., Morishita, K., Nakamura, K., Yamasaki, M. and Takiguchi, M. (2014). Evaluation of selected cytokine gene expression in colonic mucosa from dogs with idiopathic lymphocytic-plasmacytic colitis. *J Vet Med Sci:* **76** (10):1407-10.

Tani, K., Morimoto, M., Hayashi, T., Inokuma, H., Ohnishi, T., Hayashiya, S., Nomura, T., Une, S., Nakaichi, M. and Taura, Y. (2002). Evaluation of cytokine messenger RNA expression in peripheral blood mononuclear cells from dogs with canine demodicosis. *J Vet Med Sci*: **64**(6):513-518,.

vanDeuren, M., Dofferhoff, A.S.M. and van der Meer, J.W.M. (1992). Cytokines and the response to infection. *J Path*; **168**: 349-56.

vonPfeil, D.J., Cummings, B.P., Loftus, J.P., Levine, C.B., Mann, S., Downey, R.L., Griffitts, C. and Wakshlag, J.J. (2015). Evaluation of plasma inflammatory cytokine concentrations in racing sled dogs. *Can Vet J*: ***56***(12):1252.

Wang, J.E., Jorgensen, P.F., Almlof, M., Thiemermann, C., Foster, S.J., Aasen, A.O. and Solberg, R. (2000). Peptidoglycan and lipoteichoic acid from *Staphylococcus aureus* induce tumor necrosis factor alpha, interleukin 6 (IL-6), and IL-10production in both T cells and monocytes in a human whole blood model. *Infect Immun:* **68**(7):3965–3970.