White Blood Cell Count and Serum Cytokine Profile in Tropical Hardwood Workers in Kumasi

Isaac Ekow Ennin
University of Health and Allied Sciences, Ho Volta Region
Margaret Agyei Frempong
Kwame Nkrumah University of Science and Technology
Daniel Dodoo
University of Ghana
Francis A. Yeboah
Kwame Nkrumah University of Science and Technology
Raymond Saa-Eru Maalman (smaalman@gmail.com)
University of Health and Allied Sciences, Ho Volta Region

Research Article

Keywords:

Posted Date: March 30th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1412296/v1

Abstract

Background

Occupational exposure to wood dust particles has long been reported of its associated varying degrees of negative health effects due to different extractive chemicals present in the various timber species. However, tropical hardwood is also reported to have higher levels of extractive chemicals of anti-histamine, anti-oxidant and anti-inflammatory properties. In Ghana woodworkers have for years been exposed to wood dust from mixed tropical hardwood species, with little or no protective equipment such as nose masks, yet with less significant respiratory conditions. This study seeks to investigate the serum cytokine profile in tropical hardwood workers in Kumasi to provide a better understanding of the immunoregulatory pattern activated in the woodworkers.

Method

The study was carried out among woodworkers, teachers and security men located in Kumasi. A cross-sectional sampling of adult male workers was selected to participate in the study (86 woodworkers and 89 non-woodworkers). Participants donated blood collected by venepuncture into EDTA tubes and spun to separate serum for cytokine assay. Cytokines including IFN-Gamma, IL-1β, IL- 2, IL-4, IL-6, IL-10, IL-12, IL-13 and IL-17 were assayed using the Human Premixed Multi-analyte kit (R&D System, Inc. Minneapolis, USA) following the manufacturer's procedure. The cytokine levels were quantified using the Luminex*200 analyser.

Results

The mean concentration levels for the various cytokines were significantly different ($p < 0.05$) between woodworkers and non-wood workers except IL-2. There were significantly increased levels of Th1 and Th2 cytokines expressed in the woodworkers more than the non-wood workers.

Conclusions

The results from this study reveal that exposed woodworkers of mixed tropical hardwood species show a high level of Th1 and Th2 cytokines in their serum than non-woodworkers.

Introduction

Cytokines are inflammatory and immune-modulating mediators that exhibit both negative and positive regulatory effects on various target cells (Sultani et al., 2012). They are synthesized by both stationed and moving cells including mast cells, macrophages, lymphocytes and neutrophils that have been activated (Calixto et al., 2004). Infiltration of inflammatory cells such as mast cells, lymphocytes,

eosinophils, neutrophils, basophils and macrophages to the site of inflammation have been identified in inflammatory diseases such as allergic rhinitis asthma and bronchitis in temperate woodworkers (Owen 2001; Määttä, 2008). Some temperate softwood and hardwood studied have shown induction or suppression of major proinflammatory cytokines (TNF-α IL-1B, IL-6) and CD4 + effector subset cytokines Th1(IFN-γ IL-12), Th2(IL-4, IL-13) and Treg (TGF-β, IL-10) cytokines and chemokines (CCL3 CCL8, CCL11, CCL17, E-selectin) in mouse alveolar macrophage cells by the wood dust (Long et al., 2004; Määttä, et al., 2007; Bornholdt 2008). Tropical hardwood species have been shown to have high levels of useful extractive compounds such as flavonoids which present significant anti-inflammatory effects causing suppression or inhibition of proinflammatory mediators (Calixto et al., 2004; Lange 2008; Rathee et al., 2009; Toledo et al., 2013). The serum levels of various cytokines may give information on the presence, or even predictive value of inflammatory processes (Cavaillon, 2001) occurring in the woodworker. The current study investigated the serum cytokine levels induced in tropical hardwood woodworkers in Kumasi.

Methods

Characteristics of study populations

This was a cross-sectional study to investigate the cytokine levels induced in tropical hardwood workers compared with non-exposed healthy control. A total of 86 adult male woodworkers were selected by cluster and convenient sampling from Sokoban Wood Village and recruited for the study. The mean age of the woodworkers was 38.55 ± 11.12 years with a body temperature range of (34.4 – 37.3) ^{o}C. None of the woodworkers was on any medication within the period of study. A control group of 89 adult male teachers and security staff from the Kwame Nkrumah University of Science and Technology (KNUST) Basic School and KNUST Security Services who had no history of exposure to industrial air pollutants were selected for the study. The mean age of the control group was 42.74 ± 9.43 years with a body temperature range of (34.7 – 37.5) ^{oC}

Blood Sample collection

About 10ml of blood was collected from each participant by venepuncture using sterile disposable hypodermal syringes and needles. The blood was decanted 5ml each into two vacutainer tubes; a Becton Dickinson Vacutainer Hemogard serum separator tube and an ethylene diamine tetra-acetic acid (EDTA) tube. Aliquots from the EDTA tubes were taken for blood cell count. In addition, serum samples were obtained from the serum separator tubes by centrifugation at 800×g for 5 minutes at the KNUST Hospital Medical Laboratory and stored at -80^{o}C at Kumasi Centre Collaborative Research (KCCR), KNUST until used for cytokine analysis utilizing immunoassays.

Blood Cell Count

The white blood cells and platelet count were determined using the Sysmex XP-300^{TM} Automated Haematology analyser and the results printed on thermal paper. Procedures were followed as instructed

in the operational manual.

Cytokine Analysis

Serum cytokines were analysed by the multiplex ELISA technique using the Luminex Human Premixed Multi-analyte kit (R&D System, Inc. Minneapolis, USA) according to the manufacturer's instructions. The kit detected all the 9 cytokines (IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12, IL-13 IL-17) simultaneously in a single sample. Each kit contained Human Standard Cocktail, Human Premixed Microparticle Cocktail, Human Premixed Biotin-Ab Cocktail, Streptavidin–Phytoerythrin (PE) Concentrate, Diluent, Calibrator Diluent, Wash Buffer Concentrate, 96-well Microplate, Mixing Bottles Plate Sealer and a Certificate of Analysis.

All the reagents and samples were brought to room temperature (RT) and an assay was performed at RT. The 96-well filter-bottomed microplate was pre-wet by filling each well with 100µl of wash buffer and removed using a vacuum manifold designed to accommodate a microplate. In each well, 50µl of a diluted cocktail of microparticles was added to the pre-wet filtered bottom microplate. In addition, 50µl of the standard cocktail was added to the first 6 wells while to each of the rest of the wells 50µl of each sample was added and all securely covered with a foil plate. It was then incubated for 2 hours at room temperature on the horizontal orbital microplate shaker set at 500rpm. Using the vacuum manifold device, the microplate was washed by first removing the liquid, refilling each well with 100µl of wash buffer and again removing the liquid. The washing procedure was repeated 3 times. After washing, 50µl of diluted biotin antibody cocktail was added to each well and the plate was securely covered with a new foil plate sealer and incubated for 1 hour at room temperature on the shaker set at 500rpm. Again, the liquid was removed and the washing procedure was repeated three times as described above. After washing, 50µl of diluted Streptavidin-PE was added to each well, securely covered with a new foil plate sealer and incubated for 30 minutes at room temperature on the shaker set at 500rpm.

The liquid was removed and washing was repeated three times as before. The microparticles were resuspended by adding 100µl of Wash Buffer to each well. The microplate was incubated for 2 minutes at room temperature on the shaker set at 500rmp. It was read using the Luminex*200 (manufacturer: Luminex Corporation, United States of America) analyser within 30 minutes. The procedure was used to measure cytokine levels for all the samples. Cytokine concentrations were determined from standard curves prepared on each plate and expressed in picogram per millilitre (pg/ml).

Ethical Issues

The study received ethical approval from the Committee on Human Research, Publication and Ethics (CHRPE) of the School of Medical Sciences of KNUST and Komfo Anokye Teaching Hospital (KATH) Kumasi (Ref: CHRPE/AP/304/15). A written request indicating the purpose and benefits of the research was sent to the leadership of each of the participating group to seek their permission to conduct a research among their members. A durbar was held at each of the sites during which the purpose and benefits of the research was explained to them in English and the Ghanaian Language (Twi). They were

informed that participation was voluntary and participant could withdraw from the study at any time. All the participants gave informed consent form and date to return for commencement of study.

Results

Comparison of Inflammatory Cell counts between study populations

The total white blood cells, as well as the differential counts of lymphocytes, neutrophils and platelets, were compared between the woodworkers and the non-exposed workers using the student t-test. The total white blood cells, neutrophils and platelets count were significantly higher in the woodworkers than the non-exposed workers, even though the total white blood cells were only marginally significant. However, the difference in the lymphocyte counts between the woodworkers and the non-exposed workers was not significant (Table 1)

Table 1
Comparison of Inflammatory Cells Counts between Study Populations

Blood cells $x10^3$	Woodworkers Mean (SD)	Non-exposed workers Mean (SD)	p-value
Total WBC's	5.13 ± 1.351	4.66 ± 1.648	0.05
Lymphocytes	2.331 ± 0.631	2.321 ± 0.770	0.902
Neutrophils	2.228 ± 0.664	1.719 ± 1.052	< 0.001***
Platelets	203.39 ± 50.584	156.78 ± 60.498	< 0.001***
P-values *p < 0.05, **p < 0.01, ***p < 0.001 SD = standard deviation			

Comparison of Cytokine Levels Expressed in Woodworkers with Non-exposed Workers.

The level of cytokines expressed in the woodworkers and non-exposed workers are shown in Table 2. The student t-test was used to compare the mean levels of the various cytokines studied at ($p < 0.05$). Except for IL-2 which was insignificantly higher, all the other cytokines (IL-1β, IL-4, IL-6, IL-10, IL-12, IL-13, IL-17, IFN-y) studied were significantly higher in the woodworkers than the non-exposed workers.

Determining The Classes Of Cytokines Measured

The classes of cytokines measured with a significant difference in the woodworkers are shown in italics in Table 2. The major proinflammatory cytokine (IL-1β) and major CD4 + effect subsets cytokines: Th1 (IFN-y and IL-12p70), Th2 (IL-4 and IL-13) Treg (IL-10) and Th17 (IL-17) cytokines were all significantly higher in the woodworkers.

Table 2
Result of Compared Cytokine Levels between Woodworkers and Non-exposed workers

Cytokine	Woodworker (mean ± SD)	Non-exposed worker (mean ± SD)	Mean Difference	95% CI	T	p-value
IL-1β	10.26(± 2.59)	8.86(± 2.68)	1.40	0.60–2.19	3.47	0.0006***
IL-2	21.68(± 5.74)	20.17(± 5.41)	1.51	-0.15–3.17	1.79	0.0758
IL-4	27.86(± 6.27)	19.55(± 5.52)	8.31	6.54–10.07	9.29	< 0.001***
IL-6	9.34(± 3.46)	7.32(± 4.59)	2.01	0.78–3.24	3.24	0.0015**
IL-10	22.40(± 6.17)	18.63(± 6.80)	3.77	1.82–5.71	3.83	0.0002***
IL-12p70	18.29(± 4.53)	13.12(± 3.78)	5.17	3.93–6.42	8.22	< 0.001***
IL-13	7.17(± 1.77)	6.25(± 1.76)	0.93	0.40–1.45	3.47	0.0007***
IL-17A	13.30(± 2.66)	10.99(± 2.71)	2.30	1.50–3.10	5.68	< 0.001***
IFN-γ	18.63(± 4.39)	13.18(± 5.28)	5.45	3.99–6.91	7.39	< 0.001***
P-values in parentheses; *p < 0.05, **p < 0.01, ***p < 0.001, SD: Standard deviation.						

Discussion

Comparison of Inflammatory Cell Counts

Increased inflammatory cells and platelets count in the peripheral blood is an indication of inflammatory reaction in the host. In the present study, the total white blood cells, neutrophils and platelets counts were higher in the woodworkers. This is consistent with the result of Gripenbäck et al., (2005) who found a significantly increased number of neutrophils in the peripheral blood of healthy volunteers who were exposed to pinewood dust. The lymphocyte count was not significantly high in the woodworkers. This result is consistent with the findings of Gripenbäck, et al., (2005) who found significant decreased lymphocytes numbers in the peripheral blood of healthy volunteers exposed to wood dust and also found increased lymphocytes in the bronchoalveolar lavage (BAL) fluid. The increased inflammatory cells in the peripheral blood in this study suggest that the wood dust may have induced inflammatory reactions in the woodworkers.

Comparison of Cytokine levels among woodworkers and non-exposed workers

Cytokines are inflammatory and immunomodulating mediators that exhibit both negative and positive regulatory effects on various target cells (Sultani et al 2012), hence the serum levels of various cytokines may give information on the presence of inflammatory processes (Cavaillon, 2001). This study investigated the cytokine profile of the different classes of cytokines in the woodworkers:

Proinflammatory cytokines promote inflammation by activating a variety of pro-inflammatory genes, including phospholipase A2, cyclooxygenase 2 (COX-2), inducible nitric oxide synthase (iNOS), and other cytokines and chemokines to initiate inflammation (Dinarello, 2000; Apte and Voronov, 2002 Mizgerd, 2002). IL-1β and IL-6 also promote the development of T helper (Th17) cells and Th1 cell lineage (Aldrich and Vigil-Cruz 2003; Borish and Steinke, 2003). The proinflammatory cytokines (IL-1β and IL-6) were increased in the woodworkers in this study, which suggests that the wood dust induces proinflammatory cytokines and therefore may promote inflammatory reaction in the woodworkers. This observation is similar to the result of Long et al., (2004); Bornholdt et al., (2007) and Määttä, et al., (2008) in which wood dust of all the tested wood species induced proinflammatory cytokine expression in the human lung tissue and animal model studies. The present study, therefore, supports the previous observation that the wood dust induces an inflammatory response in the woodworkers

The CD4+ effector subset Th1, Th2, Th17 and Treg cytokines were higher in the woodworkers, which suggests that the wood dust may have induced Th1, Th2, Th17 and Treg immune response in the woodworkers. However, no significant changes have been reported in previous studies on the expression of Th1, Th2, Th17 and Treg cytokines in woodworkers, human tissue or animals exposed to wood dust (Määttä et al., 2005; Bornholdt 2007). Again, there were no studies reported on CD4+ effector subset cytokines induction in woodworkers exposed to tropical hardwood.

The Th1 cytokines (IFN-γ and IL-12) are known to mediate the killing of intracellular pathogens by activating monocytes and macrophages to increase their cytokines secretion and antigen presentation. However, they also resist the Th2 cell cytokine function and Th2 response (allergic response) (Moldoveanu et al., 2009; Murdock and Lloyd, 2010). Th1 cytokines also downregulate eosinophils differentiation by suppressing the development of Th2 cells in allergic inflammation (Teran, et al., 1999). This study, therefore, suggests that the increased Th1 cytokine levels observed in the woodworkers may contribute to resisting allergic inflammatory reactions that may be induced due to the increase Th2 level in the woodworkers.

This is consistent with the findings that, altering the cytokine-producing profile of Th2 cells by inducing Th1 responses is protective against Th2-related disorders such as asthma and allergy (Teixeira et al 2005). This study suggests that the Th1 response induced by the wood dust may contribute to resisting allergic response in woodworkers. This may have contributed to the normal lung function indices observed in the woodworkers in our study (not published).

The Th2 cytokines (IL-4 and IL-13) were higher in the woodworkers compared to the non-exposed workers, suggesting that the wood dust may have contributed to induce of Th2 response in the woodworkers. Th2 cytokines are important in hypersensitivity reactions and allergic immunopathology. They are known to

enhance mucus release, class switching of B cells to produce IgE, and fibrosis (Gripenbäck et al., 2005). The action of the Th2 immune response can result in IgE production, inflammation of the airways and tissue remodelling (Teran et al., 1999). Although IgE was not measured in the present study, the increased Th2 cytokines may support the high level of IgE found in a previous study of exposed tropical hardwood workers in Accra (Ennin, 2009). The increased Th2 cytokines in the woodworkers in the present study may have contributed to allergic respiratory symptoms such as sneezing and catarrh and mucous release woodworkers. This is consistent with the findings of Gripenbäck et al., (2005) and Shum et al., (2008), that elevated production of the Th2 cytokines contribute to allergic airway inflammation. The high levels of the Th1 cytokines (IFN-γ and IL-12) observed in the present study may be attributed to the high level of Th2 cytokines (IL-4) to antagonize the development of Th2 response and downregulate the Th2 inflammatory reactions in the woodworkers (Shum et al., 2008; Murdoch and Lloyd 2010).

The Th17 cytokine IL-17A was higher in the woodworkers suggesting that the wood dust may have induced a Th17 response in the woodworkers. IL-17A is known to stimulate the production of other proinflammatory cytokines and play protective roles in host resistance against pathogens at epithelial and mucosal barriers (Onishi and Gaffen, 2010; Jin and Dong, 2013). Elevated levels of IL-17A have been observed in the airways of asthmatics and are associated with neutrophil influx (Shum et al., 2008). In the present study, the IL-17A level was higher in the woodworkers this indicating that IL-17A may contribute to the induction of inflammation reaction in the woodworkers.

The woodworkers had a higher Treg cytokine (IL-10) level than the non-exposed workers suggesting an increased Treg response in the woodworkers. Activated Treg produces IL-10 which inhibits the synthesis of pro-inflammatory cytokines or suppresses their activities hence negatively modulating inflammatory response (Opal and DePalo, 2000). It also reduces airway hyper-reactivity (AHR), lung eosinophil infiltration and Th2 cytokine production which are characteristics of allergic airway inflammation. IL-10 together with IL-4 and IL-13 downregulate immunological response in the lungs (Baldacci et al., 2001; Moldoveanu et al., 2009; Murdoch and Lloyd 2010 and Erjefält 2014). The higher level of Treg cytokine in the present study suggests that the increased Treg cytokine in the woodworkers may contribute to the suppression of allergic and inflammatory reactions induced by wood dust to limit the immuno-pathological occurrence in the woodworkers.

Conclusion

The present study reveals that mixed tropical hardwood dust may contribute to inducing allergic inflammatory responses in the woodworkers which are evident by the elevated inflammatory cells, pro-inflammatory and Th2 cytokines. Moreover, the wood dust may also contribute to inhibiting allergic response and inflammatory response which may be induced in the woodworkers; this is evident by the high level of Th1 and Treg cytokines which are known to antagonize inflammatory and allergic responses.

Declarations

Ethical approval and consent to participate

The study was carried out in accordance with the guidelines and principles of the Helsinki's Declaration of research involving human participants. The basic ethical principles such as respect for persons; beneficence; and justice were adhered to in the performance of the study. The study received ethical approval from the Committee on Human Research, Publication and Ethics (CHRPE) of the School of Medical Sciences of KNUST and Komfo Anokye Teaching Hospital (KATH) Kumasi. The participation in the study was voluntary and respondents could withdraw at any stage of the data collection process. The anonymity of participants was ensured by using codes for individual answered questionnaire received.

Consent for publication

Not applicable

Availability of data and Materials

The datasets used in the study are available and can be deposited publicly if such is required.

Competing interest

The authors declare that they have no competing interests.

Funding

Partial support was obtained from the Central University Research and Training Development Fund.

Author's contributions

IEE and MAF made contributions to the conception and design of the study. **DD, FAY** and **IEE** made a substantial contribution to the study design and management of the research activities. **IEE** and **RSM** analysed the data and drafted the manuscript. All authors were involved in critical revision for important intellectual content and approved the final manuscript.

Acknowledgement

I am most indebted to Dr Samuel Dwumor of the Biostatistical Department of School of Public Health, College of Health Sciences, the University of Ghana for his assistance in the statistical analysis. I wish to express my profound gratitude to Mr Oscar of the Physiology Department of SMS KNUST and all the staff of the KNUST Hospital Medical Laboratory Department I am very grateful for their technical assistance in blood collection and haematological measurements. To Jones Amo Amponsah and Dr Bright Adu of NMIMR for assisting me and the Executives of the Sokoban Wood Workers Association and Oforikrom Wood Workers Association all of Kumasi, the Director of Security Services of KNUST, the Headteachers of KNUST Primary and Junior High Schools for organizing and encouraging their workers

and teachers to participate in the study, thank you. To all the participants who were engaged in this study, I am very grateful.

References

1. Aldrich, J.V. and Vigil-Cruz, S.C., 2003. Chemokine and cytokine modulators. Burger's medicinal chemistry and drug discovery (6th ed.) John Wiley & Sons, New York, pp.329–481.
2. Apte, R.N. and Voronov, E., 2002, Interleukin-1—a major pleiotropic cytokine in tumor–host interactions. In Seminars in cancer biology (Vol. 12, No. 4, pp. 277–290). Academic Press.
3. Baldacci, S., Omenaas, E. and Oryszczyn, M.P., 2001. Allergy markers in respiratory epidemiology. European Respiratory Journal, 17(4), pp.773–790.
4. Borish, L.C. and Steinke, J.W., 2003. 2. Cytokines and chemokines. Journal of Allergy and Clinical Immunology, *111*(2), pp. S460-S475.
5. Bornholdt, J., Hansen, J., Steiniche, T., Dictor, M., Antonsen, A., Wolff, H., Schlünssen, V., Holmila, R., Luce, D., Vogel, U. and Husgafvel-Pursiainen, K., 2008. K-ras mutations in sinonasal cancers in relation to wood dust exposure. *BMC cancer*, *8*(1), p.53.
6. Bornholdt, J., Saber A.T., Sharma A. K., Savolainen K., Vogel U. and Walin H. (2007). Inflammatory response and genotoxicity of seven wood dust in the human epithelial cell line A549. Mutat Res, 632 (1–2), pp.78–88.
7. Bornholdt, J., Saber A.T., Sharma A. K., Savolainen K., Vogel U. and Walin H. (2007). Inflammatory response and genotoxicity of seven wood dust in the human epithelia cell line A549. Mutat Res, 632 (1–2), pp.78–88.
8. Calixto, J.B., Campos, M.M., Otuki, M.F. and Santos, A.R., 2004. Anti-inflammatory compounds of plant origin. Part II. Modulation of pro-inflammatory cytokines, chemokines and adhesion molecules. Planta medica, *70*(02), pp.93–103.
9. Cavaillon, J.M., 2001. Pro-versus anti-inflammatory cytokines: myth or reality. Cellular And Molecular Biology-Paris-Wegmann-, *47*(4), pp.695–702.
10. Dinarello, C.A., 2000. Proinflammatory cytokines. Chest, 118(2), pp.503–508.
11. Ennin, E. I. 2009. *The effect of wood dust on the lung function of woodworkers at the Accra timber market*. (MPhil Thesis), College of the Health Sciences University of Ghana.
12. Erjefält, J.S., 2014. Mast cells in human airways: the culprit?. European Respiratory Review, 23(133), pp.299–307.
13. Gripenbäck, S., Lundgren, L., Eklund, A., Liden, C., Skare, L., Tornling, G. and Grunewald, J., 2005. Accumulation of eosinophils and T-lymphocytes in the lungs after exposure to pinewood dust. European Respiratory Journal, 25(1), pp.118–124.
14. Jin, W. and Dong, C., 2013. IL-17 cytokines in immunity and inflammation. Emerging microbes & infections, 2(9), p.e60.

15. Lange, J.B., 2008. *Effects of wood dust: inflammation, genotoxicity and cancer* (Doctoral dissertation, Museum Tusculanum).
16. Long, H., Shi, T., Borm, P.J., Määttä, J., Husgafvel-Pursiainen, K., Savolainen, K. and Krombach, F., 2004. ROS-mediated TNF-α and MIP-2 gene expression in alveolar macrophages exposed to pine dust. Particle and fibre toxicology, 1(1), p.3.
17. Määttä, J., 2008. *Cytokine, Chemokine, and Chemokine Receptor Expression in RAW 264.7 Mouse Macrophages and in the Lungs of Mice After Exposure to Wood Dust*. University of Kuopio.
18. Määttä, J., Haapakoski, R., Lehto, M., Leino, M., Tillander, S., Husgafvel-Pursiainen, K., Wolff, H., Savolainen, K. and Alenius, H., 2007. Immunomodulatory effects of oak dust exposure in a murine model of allergic asthma. Toxicological sciences, *99*(1), pp.260–266.
19. Määttä, J., Lehto, M., Leino, M., Tillander, S., Haapakoski, R., Majuri, M.L., Wolff, H., Rautio, S., Welling, I., Husgafvel-Pursiainen, K. and Savolainen, K., 2006. Mechanisms of particle-induced pulmonary inflammation in a mouse model: exposure to wood dust. Toxicological Sciences, *93*(1), pp.96–104.
20. Mizgerd, J.P., 2002, April. Molecular mechanisms of neutrophil recruitment elicited by bacteria in the lungs. In *Seminars in immunology* (Vol. 14, No. 2, pp. 123–132). Academic Press.
21. Moldoveanu, B., Otmishi, P., Jani, P., Walker, J., Sarmiento, X., Guardiola, J., Saad, M. and Yu, J., 2009. Inflammatory mechanisms in the lung. Journal of inflammation research, *2*, p.1.
22. Murdoch, J.R. and Lloyd, C.M., 2010. Chronic inflammation and asthma. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, *690*(1), pp.24–39.
23. Murdoch, J.R. and Lloyd, C.M., 2010. Chronic inflammation and asthma. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 690(1), pp.24–39.
24. Onishi, R.M. and Gaffen, S.L., 2010. Interleukin-17 and its target genes: mechanisms of interleukin-17 function in disease. Immunology, 129(3), pp.311–321.
25. Opal, S.M. and Depalo, V.A., 2000. Anti-inflammatory cytokines. Chest, 117(4), pp.1162–1172.
26. Owen, C., 2001. Chemokine receptors in airway disease: which receptors to target? Pulm Pharmacol Ther. 14, 193–202.
27. Rathee, P., Chaudhary, H., Rathee, S., Rathee, D., Kumar, V. and Kohli, K., 2009. Mechanism of action of flavonoids as anti-inflammatory agents: a review. Inflammation & Allergy-Drug Targets (Formerly Current Drug Targets-Inflammation & Allergy), *8*(3), pp.229–235.
28. Shum, B.O., Rolph, M.S. and Sewell, W.A., 2008. Mechanisms in allergic airway inflammation–lessons from studies in the mouse. Expert reviews in molecular medicine, *10*.
29. Sultani, M., Stringer, A.M., Bowen, J.M. and Gibson, R.J., 2012. Anti-inflammatory cytokines: important immunoregulatory factors contributing to chemotherapy-induced gastrointestinal mucositis. Chemotherapy research and practice, 2012.
30. Teixeira, L.K., Fonseca, B.P., Barboza, B.A. and Viola, J.P., 2005. The role of interferon-gamma on immune and allergic responses. Memorias do Instituto Oswaldo Cruz, *100*, pp.137–144.

31. Teran, L. M., M. Mochizuki, J. Bartels, E. L. Valencia, T. Nakajima, K. Hirai, and J.-M. Schröder. 1999. Th1- and Th2-type cytokines regulate the expression and production of eotaxin and RANTES by human lung fibroblasts. Am. J. Respir. Cell Mol. Biol. 20:777–786.
32. Toledo, A.C., Sakoda, C.P.P., Perini, A., Pinheiro, N.M., Magalhaes, R.M., Grecco, S., Tiberio, I.F.L.C., Camara, N.O., Martins, M.A., Lago, J.H.G. and Prado, C.M., 2013. Flavonone treatment reverses airway inflammation and remodelling in an asthma murine model. British journal of pharmacology, 168(7), pp.1736–1749.