

Blackcurrant anthocyanins modulate CCL11 secretion and suppress allergic airway inflammation

Odette M. Shaw^{1,2+}, Tafadzwa Nyanhanda¹⁺, Tony K. McGhie³, Jacquie L. Harper^{2,4}, and Roger D. Hurst^{1*}

¹Food & Wellness Group, The New Zealand Institute for Plant & Food Research Ltd, Palmerston North, New Zealand

²Malaghan Institute of Medical Research, Wellington, New Zealand

³Biological Chemistry & Bioactives Group, The New Zealand Institute for Plant & Food Research Ltd, Palmerston North, New Zealand

⁴Currently WelTec, Lower Hutt, New Zealand

* Corresponding Author: Dr. Roger D. Hurst

The New Zealand Institute for Plant & Food Research Ltd

Private Bag 11600,

Palmerston North 4442

New Zealand

Received: 02-Oct-2016; Revised: 26-Mar-2017; Accepted: 30-Mar-2017

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](https://doi.org/10.1002/mnfr.201600868). Please cite this article as [doi: 10.1002/mnfr.201600868](https://doi.org/10.1002/mnfr.201600868).

This article is protected by copyright. All rights reserved.

Tel: +64 6 953 7677

Fax: +64 6 351 7050

Email: roger.hurst@plantandfood.co.nz

*These authors contributed equally to this work

Abbreviations

OVA, Ovalbumin; Plant & Food Research (PFR); TNF- α , Tumour necrosis factor-alpha

Total word count: 3591

Abstract

CCL11, a chemokine, is linked to the early development of airways eosinophilia in allergic asthma. Therefore, CCL11 production is a target for abrogating eosinophilic-driven airway inflammation. Blackcurrants are high in compounds that regulate inflammation, particularly anthocyanins. In this study we investigated the effect of oral blackcurrant supplementation on allergen-induced eosinophilia and CCL11 production; we also profiled key compounds in blackcurrants that were linked to this effect. 10 mg/kg (total anthocyanins) of a commercially available, anthocyanin-rich New Zealand 'Ben Ard' blackcurrant extract ('Currantex 30') attenuated ovalbumin-induced inflammation, eosinophilia (by $52.45 \pm 38.50\%$) and CCL11 production (by $48.55 \pm 28.56\%$) in a mouse model of acute allergic lung inflammation. Ten blackcurrant polyphenolic extracts were also found to suppress CCL11 secretion by

stimulated human lung epithelial cells *in vitro*. Correlation analysis identified potential blackcurrant polyphenolic anthocyanin constituents specifically delphinidins and cyanidins, involved in CCL11 suppression. Our findings show oral supplementation with New Zealand blackcurrant is effective in reducing lung inflammation, and highlight the potential benefit of developing cultivars with specific polyphenolic profiles for the creation of functional foods with desirable biological activity.

Keywords

airway inflammation, anthocyanins, blackcurrant, CCL11, polyphenolics

1. Introduction

Allergic asthma is an inflammatory lung disease characterized by reversible airways obstruction, bronchospasm and T-helper-2-mediated infiltration of immune cells including eosinophils. [1-4]. Eosinophils contribute to mucosal inflammation and epithelial damage in the lung through the production of reactive oxygen species and cytokines, and the release of granule-derived proteins [5-7]. Damage caused by eosinophilic inflammation contributes to tissue remodelling, leading to airway hyperresponsiveness, mucus cell hyperplasia, fixed airway flow obstruction, and loss of lung function over time [8, 9]. Therefore, suppressing airways eosinophilia may be beneficial in controlling allergic asthma and lung damage.

The recruitment of eosinophils to sites of inflammation is regulated through chemokines, including the eotaxins: CCL11, CCL24 and CCL26 [1, 3, 10, 11].

Whereas CCL26 is linked with late phase persistent eosinophilia during the allergic response, CCL11 is involved in the early recruitment of eosinophils in asthmatic lung inflammation [1, 3]. This makes CCL11 a potentially useful and key target for therapy in the management of allergic airway diseases including asthma [5].

Fruits and vegetables contain numerous bioactive compounds that result in improved health and wellbeing [12-14]. Epidemiological studies present strong evidence for fruit consumption lowering both the prevalence of respiratory symptoms, and the incidence of non-specific lung diseases [15], as well as improving lung function [14, 15]. Studies also link fruit and vegetable phytochemical compounds, including anthocyanins and proanthocyanins, with the attenuation of lung inflammation [12, 16-19]. Previous research has shown that a blackcurrant extract, rich in anthocyanins, is able to suppress CCL26 secretion by human alveolar cells [20] suggesting that blackcurrant consumption could have beneficial effects on lung health.

In this study we investigated the effects of a commercially available, anthocyanin-rich New Zealand 'Ben Ard' blackcurrant extract ('Currantex 30') on CCL11 production, eosinophil recruitment and lung morphology in a murine model of allergic airways inflammation. We then evaluated 10 polyphenolic extracts from different blackcurrant cultivars as well as individual isolated pure anthocyanin glycosides for the ability to

suppress CCL11 secretion by human alveolar cells to help identify the key polyphenolic compounds in blackcurrants linked to CCL11 regulation.

2. Methods

Mice and Materials

C57BL/6J male mice were maintained in a conventional animal facility at the Malaghan Institute of Medical Research, Wellington, New Zealand. All experimental procedures were approved by the Victoria University of Wellington Animal Ethics Committee (approval number 2011R3M). 'Currantex 30', a New Zealand extract prepared from 'Ben Ard' blackcurrants, was kindly provided by Just the Berries Ltd (Palmerston North, New Zealand). Human recombinant tumour necrosis factor- α (TNF- α), Human CCL11 and Murine CCL11 DuoSet ELISA kits were purchased from R & D Systems (Minneapolis, MN, USA). Human recombinant IL-4 was purchased from PeproTech (Rocky Hill, NJ, USA). Ovalbumin (OVA), ketamine, and xylazine were purchased from Sigma (Auckland, New Zealand). Alum was from Serya (Heidelberg, Germany). The following antibodies were used for flow cytometric phenotyping of cells, including identifying eosinophils and CD4⁺ T cells: anti-mouse CD3 ϵ , CD8a, CD4, CD11b, CD11c, and Gr-1 were purchased from Biolegend (San Diego, CA, USA). Anti-mouse SiglecF, MHCII and CD45 and TMB reagent were from BD Biosciences (San Jose, CA, USA). The human alveolar epithelial cell-line A549 was purchased from the American Type Culture Collection (ATCCTM,

Manassas, VA, USA). Commercial HPLC purity (> 97%) standard isolated anthocyanin glycosides; cyanidin 3-O-glucoside chloride (kuromanin chloride) and cyanidin 3-O-rutinoside chloride (keracyanin chloride) were purchased from Extrasynthese (Genay, France) and delphinidin 3-O-glucoside (myrtillin) chloride and delphinidin 3-O-rutinoside chloride from Polyphenols Laboratories AS (Sandnes, Norway). Unless otherwise stated, all cell culture media, fetal bovine serum, supplements and buffers were purchased from Life Technologies NZ (Auckland, NZ).

Preparation of Blackcurrant Polyphenolic Extracts

Ten polyphenolic extracts were prepared and analysed (Table 1) from commercially relevant New Zealand and non-New Zealand blackcurrant cultivars; 'Murchison'; 'Ben Rua'; 'Ben Hope'; Magnus'; 'Blackadder'; 'Ben Ard', and four Plant & Food Research (PFR) selections (PFR Selections 1-4) as previously described [20].

OVA-induced Airway Inflammation Model.

Allergic airway disease was induced as previously described [21]. For the blackcurrant intervention mice were fasted overnight before being orally gavaged with 10mg/kg total anthocyanins of 'Currantex 30' 'Ben Ard' blackcurrant extract or sterile water on the day of OVA challenge and again 2 days post OVA challenge. Mice were euthanized following OVA challenge on the days indicated (Figure 1).

Immune cell infiltration and histological changes were characterised as previously described [21]. CCL11 production was quantified by mincing the left lung lobe into 500 μ L phenol red-free RPMI media and incubating at 37°C for 30 min before filtering through a 40 μ m mesh and centrifuging. CCL11 concentrations were quantified in the resulting tissue supernatants by ELISA as per the manufacturer's instructions.

In vitro CCL11 Assay

A549 human alveolar type II epithelial cells were grown and exposed to blackcurrant cultivar polyphenolic extracts or standard anthocyanins for 6 h as previously described [20] before being stimulated with 10 ng/mL IL-4 and 10 ng/mL TNF- α for 48 h. Supernatants were collected and CCL11 production was quantified by ELISA as per manufacturer's instructions.

Statistical Analysis

The *in vivo* data was analysed using one-way analysis of variance (ANOVA) with a Tukey's post hoc test or student t-test (Graphpad Prism V.5. software, San Diego, CA, USA). The *in vitro* data was analysed using one-way analysis of variance (ANOVA) using a general linear model (Minitab 15th edition statistical software

Minitab, Inc, State College, PA). Dunnett's Simultaneous Tests (corrects for multiple comparisons) were used to determine significant differences between experimental conditions and control values. Regression analysis was conducted to determine the relationship between suppression of CCL11 secretion and polyphenolic composition of the different blackcurrant cultivars.

3. Results and Discussion

Consistent with previous studies [22, 23], lung eosinophil infiltration peaked at 4-5 days following OVA challenge (Supplementary Figure 1A), and lung CCL11 concentration increased rapidly and remained significantly elevated 4 days post-OVA challenge (Supplementary Figure 1B). Comparison of haematoxylin and eosin stained lung tissue from control, OVA-challenged mice and OVA challenged mice treated with blackcurrant (Figure 1A) showed that blackcurrant extract consumption reduced OVA-induced membrane thickening (arrows), inflammatory cell infiltration (stars), and maintained normal lung tissue appearance 4 days following OVA challenge. Flow cytometry confirmed that OVA-challenged mice treated with the 'Currantex 30' 'Ben Ard' cultivar blackcurrant extract had significantly ($P < 0.05$) fewer total infiltrating cells within the lung (Figure 1B; and Supplementary Figure 1C-D). There was a non-significant trend towards a reduced CD4⁺ T cells within the mediastinal lymph node (Figure 1C), as well as non-significant trend towards decreased monocytes and neutrophil numbers (Supplementary Figure 1C-D). This led us to focus on eosinophil infiltration, and in mice that were fed blackcurrant and

challenged with OVA there was a statistically significant reduction in both eosinophils ($P < 0.05$; Figure 1D) and CCL11 levels ($P < 0.01$) when compared with OVA challenged mice that did not receive blackcurrant (Figure 1E). This was despite blackcurrant supplementation alone resulting in a non-statistically significant increase background CCL11 within the lung. The biological consequence of this trend towards higher basal CCL11 with blackcurrant consumption is unclear but is likely insignificant as it did not result in increased numbers of eosinophils (Figure 1D), or other immune cells (Figure 1B-C; and Supplementary Figure 1C-D) in the absence of the allergen. There was no change in either total or OVA-specific IgE levels (data not shown). Collectively these results indicate that 'Ben Ard' blackcurrant anthocyanin extract consumption attenuates allergic airway inflammation primarily through a reduced number of infiltrating eosinophils in association with CCL11 inhibition. Other studies have shown that plant compounds, especially polyphenols, can influence the allergic immune responses during allergen sensitization and during allergen re-exposure [14]. Therefore, we further explored the effects of polyphenolic extracts from different New Zealand blackcurrant cultivars on CCL11 secretion by human alveolar epithelial cells.

We found that all the blackcurrant fruit polyphenolic extracts significantly suppressed CCL11 production by IL-4/TNF- α stimulated lung epithelial cells *in vitro* (Figure 2A), with 'Magnus' and PFR selections 2 and 3 extracts being the most effective. Regression analysis compared the percentage CCL11 suppression against the

individual polyphenolic compounds present in the blackcurrant cultivar extracts (Table 1). Regression analysis identified total delphinidins as the most positively correlated class of compounds (Table 2) as well as significant positive correlations for delphinidin 3-O-glucoside ($r=0.450$) and delphinidin 3-O-rutinoside ($r=0.541$; Table 2). To test whether the observed anti-inflammatory activity of the different blackcurrant cultivars was associated with the polyphenolic composition, individual anthocyanin compounds were tested for the ability to suppress IL-4/TNF α -induced CCL11 production by lung epithelial cells. Delphinidin 3-O-rutinoside mediated a 50% inhibition of CCL11 secretion, ($p<0.001$) whereas delphinidin 3-O-glucoside mediated 30% inhibition ($p<0.001$; Figure 2B). There were also significant negative correlations with total cyanidins, total phenolics and total flavonols (Table 2). High concentrations of isolated cyanidin 3-O-rutinoside increased CCL11 secretion, ($p<0.001$), which was consistent with the negative correlation ($r=-0.477$) between cyanidin 3-O-rutinoside levels in the blackcurrant fruit extracts and CCL11 inhibition (Table 2). At lower concentrations neither of the pure cyanidin anthocyanin glycosides had an effect on CCL11 secretion (Figure 2B). This suggests that the statistically significant, but low positive correlation ($r=0.225$) we identified between cyanidin 3-O-glucoside and CCL11 inhibition in the cultivars is unlikely to be an important component for modulating CCL11 production by anthocyanins. Overall, while the pure anthocyanins had a consistent effect on CCL11 production, the effects of the individual compounds was less than that observed was seen with whole fruit extracts. Collectively, these results indicate that polyphenolic composition overall is capable of mediating the observed CCL11 inhibition. Deeper correlation analysis

(Table 2) showed that having a high delphinidin to low cyanidin anthocyanin glycoside ratio in the fruit resulted in increased inhibition of CCL11 ($r=0.788$; $p<0.01$). This pattern of suppressive activity was similar to that previously reported for CCL26 suppression [20]. To remain physiologically relevant, we used molar concentrations of the individual anthocyanins that were mid-range equivalent to the amounts of the individual components in the extracts. While it is possible we would have seen stronger dose response effects for the individual anthocyanins if we had increased the concentration or used a wider range of concentrations, this would have been less biologically relevant to the levels of anthocyanins found in the whole fruit. This, and our correlation analysis results highlight that the ratio of polyphenolics within the fruit is an important determinant for immune modulation rather than each individual phenolic compound alone and that there are more complex chemical interactions occurring from the diverse different polyphenolic components of the blackcurrant extracts leading to the suppression of CCL11 we observe. However, the data indicates that blackcurrant cultivars high in delphinidin anthocyanin glycosides will likely exhibit beneficial biological functionality in allergic lung inflammation, namely decreased CCL11 production, eosinophil infiltration and reduced tissue damage.

Concluding remarks

Our findings provide evidence that anthocyanin-rich New Zealand blackcurrant extract consumption can reduce eosinophil recruitment and alleviates eosinophilic-driven airway inflammation in allergic asthma, in part, by moderating CCL11

production. Furthermore, we have shown that the ratio of polyphenolics within the fruit is an important determinant for immune modulation rather than an individual phenolic compound. Fruit extracts with a high delphinidin to low cyanidin anthocyanin glycoside ratio have the potential to be most effective at alleviating airways inflammation and inflammatory cell recruitment. While this study provides part of the potential mechanism by which dietary polyphenols can modulate immune function, more work needs to be done to fully understand the complex interactions between multiple compounds and the immune system to identify the exact mechanisms by which blackcurrant modulates the immune response to allergen-mediated inflammation. These findings illustrate the potential benefits of blackcurrant consumption for lung health, and demonstrate that targeted breeding could be used to develop cultivars with specific polyphenolic profiles designed to be more effective at attenuating lung inflammation.

mnfr201600868-sup-0001-SuppMat-Fig1.JPG

Supplementary Figure 1: Time course of ovalbumin-induced airway eosinophil infiltration and CCL11 production and blackcurrant effect on monocyte and neutrophil lung infiltration. Mice were primed with OVA/Alum i.p. and then challenged 7 days later with OVA i.n. (Day 0). (A) Eosinophil numbers in bronchioalveolar lavage fluid, and (B) CCL11 production were determined at different time points (n=5 per time point). *p<0.05 compared to 0 h (One-way ANOVA with Tukey's Post Hoc test). For the intervention study mice were primed with OVA/Alum

i.p. and then challenged 7 days later with OVA i.n. these mice were orally gavaged with 10 mg/kg of a commercially available New Zealand anthocyanin-rich blackcurrant extract (BC) 1h before OVA challenge and again 2 days post-challenge. (C) Neutrophils and (D) monocytes within the bronchioalveolar lavage fluid were determined 4 days post-OVA challenge (n=10 per intervention group). ** $p < 0.01$ compared to OVA challenge (One-way ANOVA with Tukey's Post Hoc test).

Author Contributions

OMS designed, performed, analysed and interpreted the *in vivo* (animal) studies, wrote and contributed to the editing of the manuscript; TN designed, performed, analysed and interpreted the *in vitro* (cell culture) studies and contributed to the editing of the manuscript; TKM prepared the phenolic blackcurrant extracts, analysed and interpreted the chemical analysis, and contributed to the editing of the manuscript; JLH designed, analysed and interpreted the *in vivo* (animal) studies, wrote and contributed to the editing of the manuscript; RDH secured the funding and lead the programme supporting this research, RDH also designed, analysed and interpreted the *in vitro* (cell culture) studies, wrote and contributed to the editing of the manuscript.

Acknowledgements

This work was funded by the Ministry for Business, Innovation and Education (New Zealand), contract number C11X1002 awarded to R. D Hurst at The New Zealand Institute for Plant & Food Research Ltd. The authors would like to thank Michaela Walton for her assistance with the *in vivo* experiments.

Conflict of Interest

None of the authors declare a conflict of interest.

5 References

- [1] Djukanovic, R., Wilson, J. W., Britten, K. M., Wilson, S. J., *et al.*, Effect of an inhaled corticosteroid on airway inflammation and symptoms in asthma. *Am Rev Respir Dis* 1992, 145, 669-674.
- [2] Cockcroft, D. W., Davis, B. E., Mechanisms of airway hyperresponsiveness. *J Allergy Clin Immunol* 2006, 118, 551-559; quiz 560-551.
- [3] Komiya, A., Nagase, H., Yamada, H., Sekiya, T., *et al.*, Concerted expression of eotaxin-1, eotaxin-2, and eotaxin-3 in human bronchial epithelial cells. *Cell Immunol* 2003, 225, 91-100.
- [4] Leung, D. Y., Molecular basis of allergic diseases. *Mol Genet Metab* 1998, 63, 157-167.

- [5] Gleich, G. J., Mechanisms of eosinophil-associated inflammation. *J Allergy Clin Immunol* 2000, 105, 651-663.
- [6] Weller, P. F., The immunobiology of eosinophils. *N Engl J Med* 1991, 324, 1110-1118.
- [7] Trivedi, S. G., Lloyd, C. M., Eosinophils in the pathogenesis of allergic airways disease. *Cell Mol Life Sci* 2007, 64, 1269-1289.
- [8] Holgate, S. T., Epithelium dysfunction in asthma. *J Allergy Clin Immunol* 2007, 120, 1233-1244; quiz 1245-1236.
- [9] Bergeron, C., Al-Ramli, W., Hamid, Q., Remodeling in asthma. *Proc Am Thorac Soc* 2009, 6, 301-305.
- [10] Rokudai, A., Terui, Y., Kuniyoshi, R., Mishima, Y., *et al.*, Differential regulation of eotaxin-1/CCL11 and eotaxin-3/CCL26 production by the TNF-alpha and IL-4 stimulated human lung fibroblast. *Biol Pharm Bull* 2006, 29, 1102-1109.
- [11] Matsukura, S., Stellato, C., Plitt, J. R., Bickel, C., *et al.*, Activation of eotaxin gene transcription by NF-kappa B and STAT6 in human airway epithelial cells. *J Immunol* 1999, 163, 6876-6883.
- [12] Arts, I. C., Hollman, P. C., Polyphenols and disease risk in epidemiologic studies. *Am J Clin Nutr* 2005, 81, 317S-325S.

- [13] Nyanhanda, T., Gould, E. M., Hurst, R. D., Plant-derived foods for the attenuation of allergic airway inflammation. *Curr Pharm Des* 2014, 20, 869-878.
- [14] Singh, A., Holvoet, S., Mercenier, A., Dietary polyphenols in the prevention and treatment of allergic diseases. *Clin Exp Allergy* 2011, 41, 1346-1359.
- [15] Rosenlund, H., Kull, I., Pershagen, G., Wolk, A., *et al.*, Fruit and vegetable consumption in relation to allergy: disease-related modification of consumption? *J Allergy Clin Immunol* 2011, 127, 1219-1225.
- [16] Scalbert, A., Johnson, I. T., Saltmarsh, M., Polyphenols: antioxidants and beyond. *Am J Clin Nutr* 2005, 81, 215S-217S.
- [17] Garbacki, N., Kinet, M., Nuscgens, B., Desmecht, D., Damas, J., Proanthocyanidins, from *Ribes nigrum* leaves, reduce endothelial adhesion molecules ICAM-1 and VCAM-1. *J Inflamm (Lond)* 2005, 2, 9.
- [18] Hurst, S. M., McGhie, T. K., Cooney, J. M., Jensen, D. J., *et al.*, Blackcurrant proanthocyanidins augment IFN-gamma-induced suppression of IL-4 stimulated CCL26 secretion in alveolar epithelial cells. *Mol Nutr Food Res* 2010, 54 Suppl 2, S159-170.
- [19] Park, S. J., Shin, W. H., Seo, J. W., Kim, E. J., Anthocyanins inhibit airway inflammation and hyperresponsiveness in a murine asthma model. *Food Chem Toxicol* 2007, 45, 1459-1467.

- [20] Nyanhanda, T., Gould, E. M., McGhie, T., Shaw, O. M., *et al.*, Blackcurrant cultivar polyphenolic extracts suppress CCL26 secretion from alveolar epithelial cells. *Food Funct* 2014, 5, 671-677.
- [21] Shaw, O. M., Harper, J. L., An efficient single prime protocol for the induction of antigen-induced airways inflammation. *Journal of Immunological Methods* 2013, 395, 79-82.
- [22] Shaw, O. M., Harper, J. L., An efficient single prime protocol for the induction of antigen-induced airways inflammation. *Journal of Immunological Methods* 2013, 395, 79-82.
- [23] Nials, A. T., Uddin, S., Mouse models of allergic asthma: acute and chronic allergen challenge. *Dis Model Mech* 2008, 1, 213-220.

Figure 1: Blackcurrant extract suppresses Ovalbumin-induced airway inflammation, eosinophil infiltration and CCL11 production.

Mice were primed with ovalbumin (OVA)/Alum i.p. and then challenged 7 days later with OVA i.n. Mice were orally gavaged with 10 mg/kg of 'Currantex 30' New Zealand anthocyanin-rich 'Ben Ard' blackcurrant extract (BC) 1h before OVA challenge and again 2 days post-challenge. (A) Representative haematoxylin and eosin stained lung tissue from naïve, OVA-challenged and OVA-challenged mice treated with blackcurrant. Magnification 20x (top) and 40x (bottom)

Arrow=membrane thickening; stars=cell infiltration. (B) Total bronchioalveolar lavage fluid cells, (C) mediastinal lymph node CD4+ T cells, (D) bronchioalveolar lavage fluid eosinophils, and (E) CCL11 production in lung tissue were determined 4 days post-OVA challenge (n=10 per intervention group). *p<0.05, ** p<0.01, *** p<0.001 compared to OVA challenge (One-way ANOVA with Tukey's Post Hoc test).

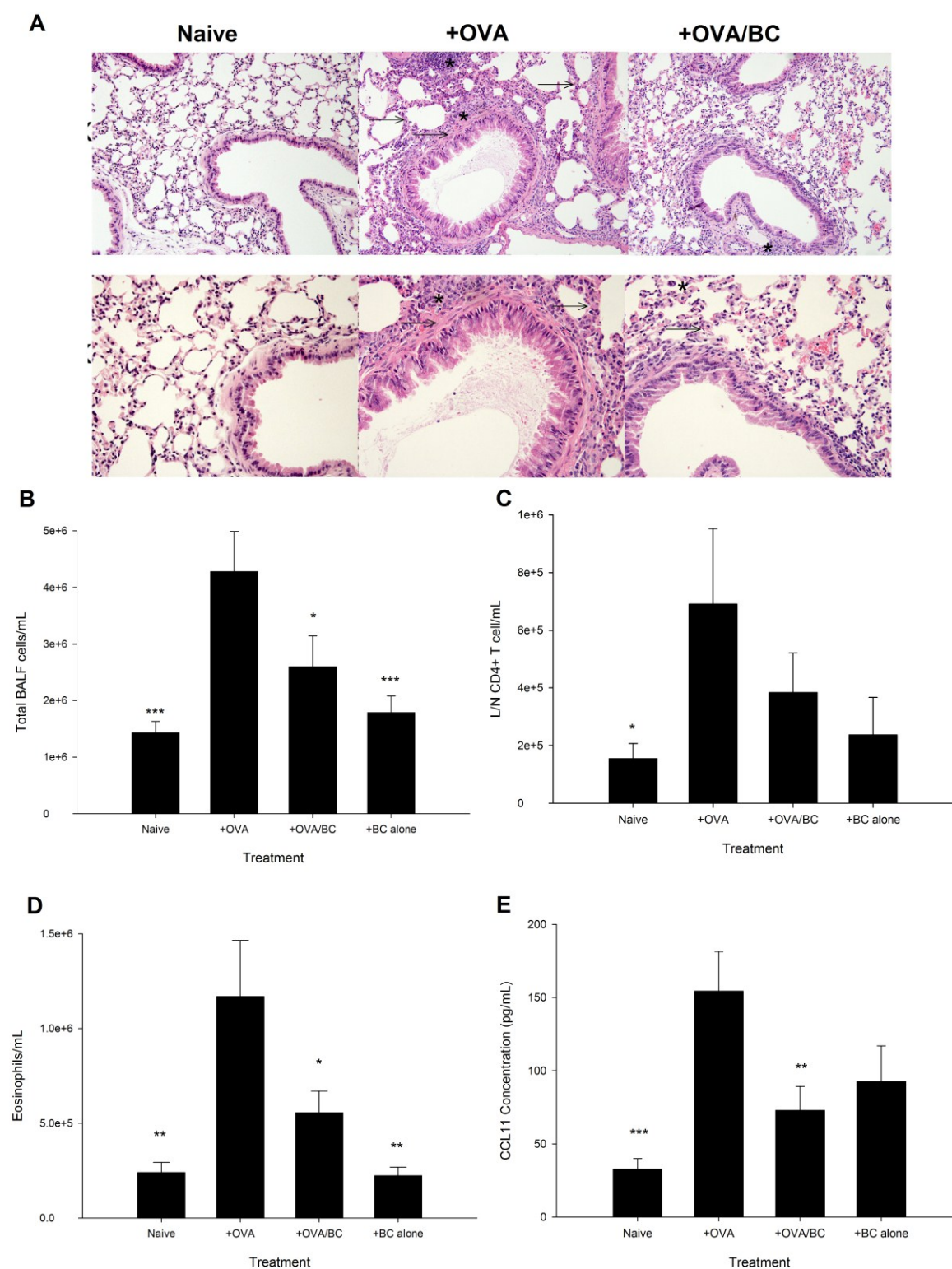
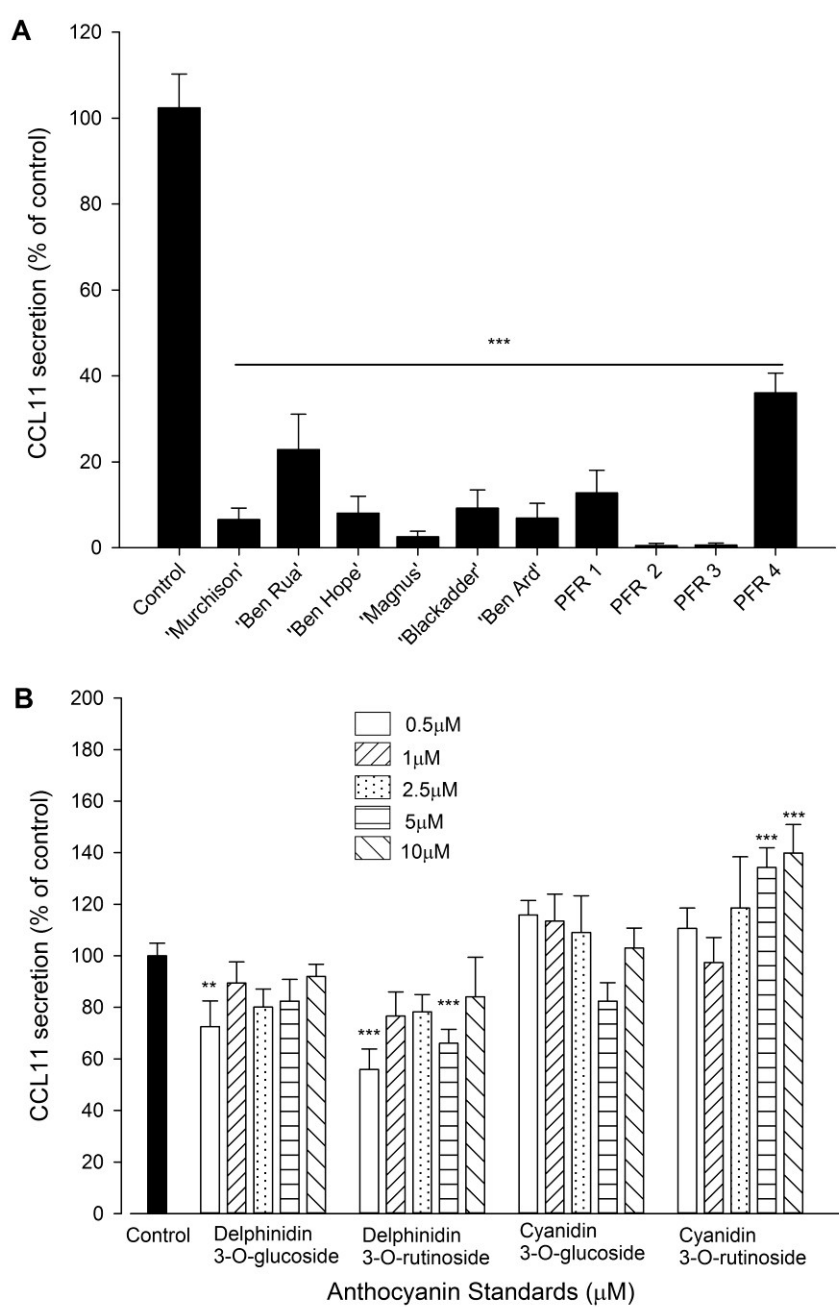


Figure 2: Effect of different blackcurrant cultivar polyphenolic extracts and pure anthocyanin glycosides on CCL11 secretion from A549 cells. Alveolar epithelial cells were pre-incubated for 6 h with (A) 10 µg/mL blackcurrant cultivar extracts or (B) 0-10 µM individual anthocyanin glycosides then stimulated with 10 ng/mL IL-4 and 10 ng/mL TNF-α for 48 h. Supernatants were collected and CCL11 measured. Results are expressed as mean ± SEM CCL11 secretion as a percentage of the controls (100%, n=6-8 individual replicates). *p<0.05, ** p<0.01, *** p<0.001 compared to control.



Graphical Abstract

We investigated blackcurrant in a model of allergic lung inflammation and found New Zealand 'Ben Ard' blackcurrant ('Currantex 30') reduced eosinophilia and CCL11 production. In stimulated human lung epithelial cells blackcurrant extracts also suppressed CCL11 and we identified the potential polyphenolic anthocyanins involved. Our findings highlight the potential benefit of specific polyphenolic-containing blackcurrant cultivars for reducing lung inflammation.

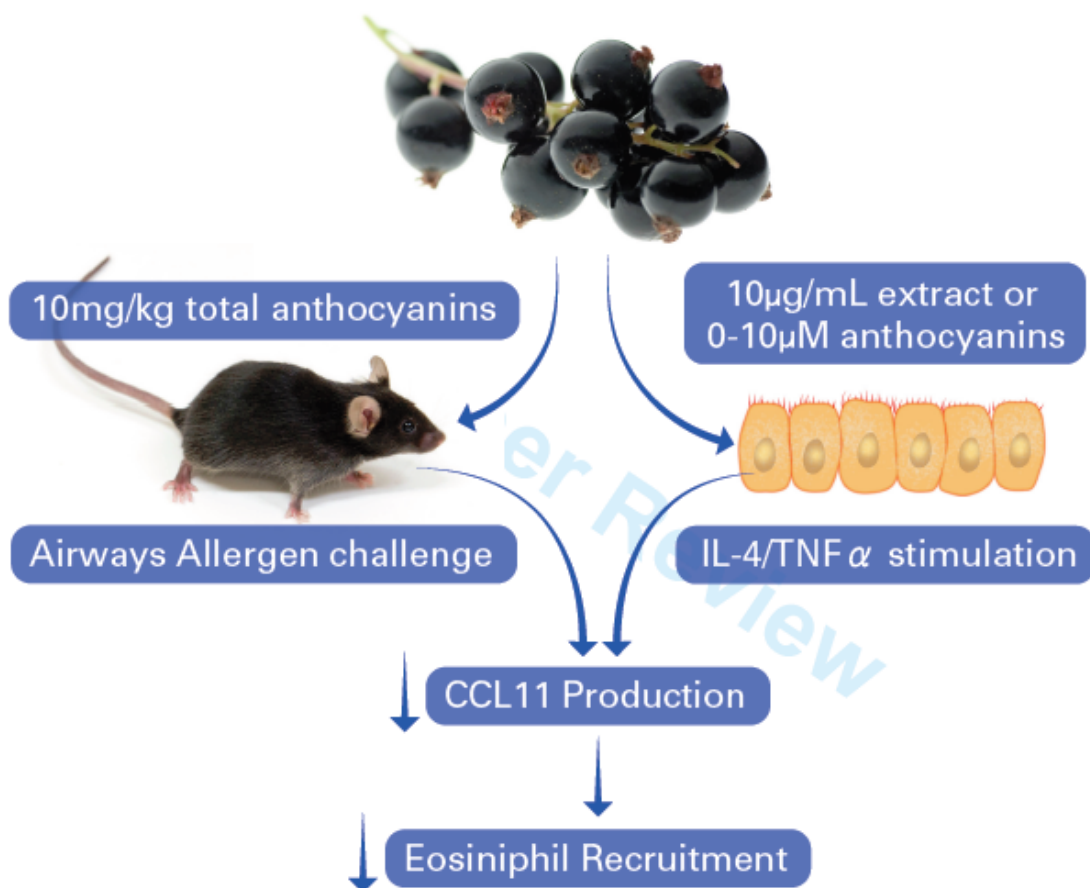


Table 1: Polyphenolic composition of blackcurrant cultivar extracts.

Polyphenolics were identified and quantified (mg/g fresh weight fruit) in each blackcurrant cultivar extract using reversed-phase HPLC and calibrated against cyanidin 3-O-glucoside and quercetin 3-O-rutinoside standards.

Cultivar	Delphinidin 3-O-glucoside	Delphinidin 3-O-rutinoside	Cyanidin 3-O-glucoside	Cyanidin 3-O-rutinoside	Petunidin 3-O-glucoside	Petunidin 3-O-rutinoside	Peonidin 3-O-rutinoside	Delphinidin 3-O-coumaroyl glucoside	Cyanidin 3-O-coumaroyl glucoside	Myricetin 3-O-rutinoside	Myricetin 3-O-glucoside	Quercetin 3-O-rutinoside	Quercetin 3-O-glucoside	Total Anthocyanins	Total Flavonols	Total Phenolics
'Murчисон'	11	108	10	167	0.7	1.5	0.7	1.7	0.9	13.3	8.3	7.8	5.5	302	34.9	938
'Берн Рубин'	3	74	3	195	1.3	10.3	3.9	2	1	21.1	8.3	11.6	4.9	293	45.9	906
'Берн Хоуп'	19	144	15	135	1	2.8	1.4	1.4	1.1	8.5	10.5	4.8	3.6	321	27.4	914
'Магнус'	34	114	19	121	0.8	1.3	0.7	1.1	0.5	6.9	4.7	5.9	3.1	292	20.6	889
'Блаккадде'	23	146	11	145	0.9	1.2	0.5	2.7	1	12.7	10.3	5.5	2.1	331	30.7	988

r'																
'Be n Ard'	36	145	15	13 5	1.2	2.9	2	1.6	0.8	12	6.4	5.2	2.8	339	26. 4	94 7
PFR Sele ctio n 1	24	112	12	12 0	0.8	2.7	1.2	1.1	0.6	8.9	5.6	8.4	6.2	274	29. 1	87 0
PFR Sele ctio n 2	24	122	12	14 0	0.9	0.8	0.3	2.1	1.3	9.2	7.5	6.4	1.7	304	24. 8	89 7
PFR Sele ctio n 3	14	118	6	89	0.7	4.1	1.4	1	0.8	10	3.9	8.1	3.5	235	25. 5	74 2
PFR Sele ctio n 4	8	68	11	17 3	1.3	2.6	1.4	2.1	0.7	19. 9	14. 4	9.6	5	268	49	10 39

Table 2: Correlation coefficient analysis of inhibition of CCL11 secretion and polyphenolic composition of extracts from different blackcurrant cultivars.

Regression analysis was conducted to determine the relationship between percentage suppression of CCL11 secretion and the polyphenolic composition of the different blackcurrant cultivars * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

Polyphenolic Compound	Pearson Correlation Coefficient (r) CCL11
Delphinidin 3- <i>O</i> -glucoside	0.450 ^{***}
Delphinidin 3- <i>O</i> -rutinoside	0.541 ^{***}
Cyanidin 3- <i>O</i> -glucoside	0.225 [*]
Cyanidin 3- <i>O</i> -rutinoside	-0.477 ^{***}
Myricetin 3- <i>O</i> -rutinoside	-0.584 ^{***}
Myricetin 3- <i>O</i> -glucoside	-0.518 ^{***}
Petunidin 3- <i>O</i> -glucoside	-0.539 ^{***}
Petunidin 3- <i>O</i> -rutinoside	-0.289 ^{**}
Peonidin 3- <i>O</i> -rutinoside	-0.336 ^{**}
Delphinidin 3- <i>O</i> -coumaroyl glucoside	-0.258 [*]
Cyanidin 3- <i>O</i> -coumaroyl glucoside	0.134
Quercetin 3- <i>O</i> -glucoside	-0.377 ^{***}
Quercetin 3- <i>O</i> -rutinoside	-0.474 ^{***}
Total Delphinidins	0.551 ^{***}
Total Cyanidins	-0.461 ^{***}
Total Cyanidins:Total Delphinidins	0.788 ^{**}
Total Anthocyanins	0.132

Total Phenolics	-0.29 ^{***}
Total Flavonols	-0.651 ^{***}

Regression analysis was conducted to determine the relationship between percentage suppression of CCL11 secretion and the polyphenolic composition of the different blackcurrant cultivars * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.