Mol Cell Toxicol (2010) 6:313-319
DOI 10.1007/s13273-010-0042-1

ORIGINAL PAPER

Correlation between nutrition intake and gene expression profiles in children with asthma

Jae-Eun Pie[1,*], Yu-Ri Kim[2,*], In-Kyoung Kim[2], Sang-Hui Seo[2], Seung Ho Lee[2], Hee Ra Lee[2], Young Yoo[3], Ji Tae Chung[3], Jong Pil Youn[4], MoonJu Oh[4], Seung Yong Hwang[4] & Meyoung-Kon Kim[2]

Received: 10 May 2010 / Accepted: 19 June 2010

Abstract Asthma is a complex inflammatory disease and its prevalence has increased worldwide, especially in young children. In this study, we used a '24-hour recall method' to identify differences between children with and without asthma in energy intake and energy-adjusted nutrition intake. We also performed reverse transcription-polymerase chain reaction (RT-PCR) with pathway-targeted arrays (RT2 Profiler™ PCR Array) to investigate the expression profiles of chemokines and cytokines in children with asthma. The intake of vitamin C in mild and moderate asthma was significantly higher than that in healthy controls. Vitamin E intake in the mild asthma group was also significantly higher. Twenty-three genes were expressed at higher levels in severe asthma compared with healthy controls. Using the human Th1-Th2-Th3 PCR Array, we found 17 genes were upregulated in severe asthma, including the Th2-related genes *CCL7*, *IL13*, and *CCL-11* (*eotaxin*). These PCR Array results revealed that the genes that were most profoundly increased in asthma encoded for key proinflammatory and chemotactic molecules. Our observations lead us to speculate that the interaction between gene expression and dietary intake is important for the development of asthma.

[1]Department of Food and Nutrition, College of Science and Engineering, Anyang University, Anyang, Korea
[2]Department of Biochemistry & Molecular Biology, Korea University, College of Medicine, Seoul, Korea
[3]Research Center for Environment and Health, Korea University Anam Hospital, Seoul, Korea
[4]GenoCheck CO., LTD., Hanyang University, Ansan, Korea
*These authors contributed equally to this work
Correspondence and requests for materials should be addressed to M. K. Kim (✉jerrykim@korea.ac.kr)

Keywords Asthma, Children, Nutrition intake, Gene expression profile, PCR array

Asthma is one of the most common chronic airway inflammatory diseases and is the cause of serious morbidity and mortality in childhood. The prevalence of asthma has been increasing in Korea and many other countries[1-3], leading to a substantial increase in public expenditure on this disease. In Korea, the prevalence of asthma in children is approximately 18.6% and the estimated cost for treatment in 2005 was approximately 77 million US dollars. The a etiology of asthma is complex, involving environmental factors such as allergens, air pollution, tobacco smoke, and dietary pattern, as well as genetic compounds.

The rapid increase in the prevalence of asthma in western countries may be related to the substantial dietary changes in these countries over the same time period. For example, the consumption of fast food, which contains high levels of fat and protein and low vitamin levels, has been associated with increased asthma prevalence in childhood[4-6]. Also, diets high in fruits and vegetables containing antioxidants, such as vitamin C, vitamin E, beta-carotene and flavonoid, have been associated with a reducedprevalence of asthma[7,8]. Conversely, a diet low in vitamin C is a known risk factor for asthma[9]. Moreover, maternal vitamin E intake during pregnancy has been associated with a decreased prevalence of childhood asthma[10,11]. Since asthma is known to be a complex immunological disease, a western-style diet may be related to increased asthma prevalence due to its capacity to modulate the immune response.

Asthma is caused by an aberrant immune response

Table 1. Characteristics of the study subjects.

	Healthy control (n=12)	Children with asthma		
		Mild (n=18)	Moderate (n=8)	Severe (n=5)
Age (yr)	6.6±0.3	7.8±1.2	8.2±1.5	9.7±3.1
Sex (F/M)	7/5	11/7	4/4	3/2
FEV1 %p	99.52±9.92	94.99±12.54	91.43±9.89	76.24±8.15**
Height (cm)	123.2±6.78	126.2±6.67	127.9±6.41	140.6±22.17
Weight (kg)	26.2±4.21	28.4±4.47	25.7±3.08	37.0±17.3**

Data expressed as mean±SD.
** indicates a statistically significant difference, $^{**}P<0.01$ *vs* control (One-way ANOVA).

Table 2. Comparison of energy intake and energy-adjusted nutrition intake in the study participants.

	Healthy control	Asthma		
		Mild	Moderate	Severe
Energy (kcal)	1565.9±340.6	1733.8±624.3	1489.8±280.9	1659.7±591.8
Protein (g)	73.6±47.2	65.5±35.3	53.9±12.0	69.1±35.2
Fat (g)	51.8±18.9	52.1±23.8	41.5±10.0	50.4±23.6
Carbohydrate (g)	214.8±54.3	253.6±78.1	224.3±47.7	241.5±71.6
Vitamin A (μgRE)	697.8±272.1	677.8±450.4	604.9±291.1	640.2±266.9
Vitamin C (mg)	50.1±19.3	92.8±55.4**	86.2±36.0*	50.1±20.4
Vitamin E (mg)	10.8±2.9	16.3±7.8**	10.5±3.1	11.7±4.1
Thiamin (mg)	0.92±0.34	1.15±0.72	0.96±0.30	1.29±0.75
Riboflavin (mg)	1.11±0.45	1.20±0.55	1.06±0.31	1.20±0.53
Niacin (mg)	12.0±3.96	14.1±8.25	10.7±2.66	13.2±9.11
Vitamin B6 (mg)	1.46±0.46	2.00±0.95	1.45±0.58	1.66±0.79
Calcium (mg)	544.1±253.2	582.8±340.8	477.3±210.9	581.9±269.6
Iron (mg)	9.43±3.02	11.5±6.12	9.49±2.03	10.6±5.94
Zinc (mg)	7.19±1.83	9.99±9.29	6.71±1.46	7.61±3.34
Sodium (mg)	2862.4±913.5	3628.5±2185.6	2887.8±717.3	3634.3±1803.5
Cholesterol (mg)	351.3±168.7	377.3±306.9	295.5±116.4	396.5±277.2
Fatty acid (g)	31.2±15.2	36.0±19.6	23.0±7.52	35.9±24.0
Fiber (g)	14.1±4.35	16.4±9.30	13.8±4.10	13.7±6.28

Data expressed as mean±SD.
*, ** indicates statistically significant differences, $^{*}P<0.05$, $^{**}P<0.01$ *vs* control (One-way ANOVA).

against allergens and environmental pollutants, and involves inflammatory cells such as T cells, eosinophils, mast cells, and neutrophils[12,13]. These cells release many cytokines and chemokines, and the expression levels of and genetic polymorphisms in these mediators have been associated with disease severity and response to treatment. Cytokines are immunomodulatory glycoproteins that are known to regulate chemotaxis, immunoglobulin isotype switching, cell differentiation, and cell-cell signaling. To date, more than 30 different cytokines responsiblein the pathogenesis asthma have been identified[13]. Chemokines are a large family of chemotactic proteins whose expression in regulated by cytokines in a variety of inflammatory conditions including rheumatoid arthritis, allergy and asthma. Chemokines are divided into 4 subfamilies, including CC, CXC, C, and CX3C, based on the spacing of their first two cysteine residues[14-16]. Several studies have shown the importance of chemokines in the regulation of asthmatic responses[17,18]. However, the genetic and molecular mechanisms that regulate the gene expression patterns that determine the severity of asthma, especially in childhood, remain unclear. Therefore, analysis of gene expression profiles, focusing on differences in expression levels of specific inflammation-related genes and their relation to asthma severity, may be useful to understand important molecular mechanisms inasthma.

In this study, we used a new high-throughput technique, RT^2 Profiler^{TM} PCR array, which combines features of SYBR Green real-time PCR and microarrays. The PCR array focuses on specific pathways and diseases and can be used to identify gene expression profiles in children with asthma of varying degrees of

Table 3. Differentially-regulated inflammatory cytokine and receptor genes in PBMC of children with asthma.

Gene name	Mild		Moderate		Severe	
	Fold change	*P*-value	Fold change	*P*-value	Fold change	*P*-value
Chemokine genes						
CXCL12	−1.85	0.0009	1.31	0.0056	3.36	0.0005
CXCL13	−1.09	NS	6.21	0.0003	33.03	0.0002
CCL7	−1.09	NS	1.33	0.0073	6.72	0.0002
CCL11	−1.80	0.0007	1.90	0.0025	4.36	0.0006
CCL13	−1.09	NS	7.81	0.0001	4.69	0.0012
CCL15	−1.59	0.0006	−1.10	0.0135	6.66	0.0001
CCL17	−1.09	NS	2.24	0.0029	5.25	0.0004
CCL21	−1.04	NS	1.33	0.0073	10.68	0.0002
CCL20	1.12	NS	1.03	NS	4.41	0.0011
CCL23	1.26	0.0132	4.44	0.0006	4.09	0.0003
CCL25	−1.14	0.0166	1.06	NS	6.06	0.0002
CCL26	−1.09	NS	2.90	0.0015	7.11	0.0008
Cytokine genes						
IL1F7	1.80	0.0055	1.92	0.0103	3.55	0.0014
IL1F8	−1.09	NS	1.33	0.0073	14.96	0.0002
IL1F9	−1.09	NS	1.33	0.0073	9.76	0.0003
IL1A	−1.09	NS	5.19	0.0004	5.26	0.0004
IL17C	2.00	0.0014	3.09	0.0012	4.67	0.0004
SPP1	1.52	0.0067	2.97	0.0008	1.54	0.0138
IL9	1.63	0.0031	3.28	0.0002	4.65	0.0001
Other inflammation-related genes						
CRP	−1.09	NS	3.23	0.0008	7.82	0.0003
C3	−1.09	NS	1.33	0.0073	5.37	0.0003

NS, non significant

severity. The aim of the present study was to test whether there is a relationship between asthma severity in children and dietary intake and immune gene expression profiles. This study will provide insights into the molecular mechanisms of asthma exacerbation and may ultimately be useful for developing new treatments for pediatric asthma.

Daily nutrient intake in children with and without asthma

The mean daily nutrient intakes in children with and without asthma are presented in Table 2. There were no significant differences between those with and without asthma in the intake of energy, protein, fat, and carbohydrate. There were also no differences in the energy-adjusted intake of B vitamins, including thiamin (B_1), riboflavin (B_2), niacin (B_3) and vitamin B_6, and of minerals including calcium, iron, zinc, and sodium among controls and mild, moderate and severe as asthmatic. However, vitamin C intake was significantly higher in mild (92.8 ± 55.4, $P < 0.01$) and moderate (86.2 ± 36.0, $P < 0.05$) asthmatics compared to healthy controls. Vitamin E intake was significantly higher in mild (16.3 ± 7.8, $P < 0.01$) asthma compared to moderate and severe asthmatics, and healthy controls. Based on these results, we speculate that children with mild asthma have ingested greater amounts of antioxidant-rich foods such as fruits and vegetables as a means to improve their symptoms of asthma.

Gene expression patterns of children with asthma by using inflammatory cytokine and receptor PCR array

Among the 84 transcripts in the PCR array of the inflammatory cytokines and receptors, 4, 14, and 23 genes were upregulated in mild, moderate, and severe asthma (fold change ≥ 2.0 and *P*-value < 0.05), respectively, compared to healthy controls. As shown in Table 3, chemokine genes such as *chemokine* (*C-X-C motif*) *ligand 13* (*CXCL13*), *CXCL12*, *chemokine* (*C-C motif*) *ligand 17* (*CCL7*), *CCL11*, *CCL13*, and *CCL15* were highly upregulated in children with asthma compared with healthy control. In particular, *CXCL13* in severe asthma was the most strongly upregulated gene (33.03-fold) among all three asthma groups. In addition to chemokines, cytokines belonging to interleukin 1 family including *IL1F8*, *IL1F9*, and *IL1A* were upregulated in the moderate and severe asthma group (Table 3). The fold-change values for several genes

Table 4. Differentially-regulated Human Th1-Th2-Th3 genes in PBMC of children with asthma.

Gene name	Official symbol	Fold change		
		Mild	Moderate	Severe
Th2 cytokines and related genes				
Chemokine (C-C motif) ligand 11 (eotaxin-1)	CCL11	−1.19	1.53	13.74
Interleukin 13	IL13	1.06	−1.62	4.42
Interleukin 10	IL10	−1.19	3.83	3.78
Chemokine (C-C motif) ligand 7 (MCP-3)	CCL7	−1.19	3.15	3.58
Interleukin 4	IL4	−1.19	1.48	2.99
Interleukin 5	IL5	−1.14	1.61	2.33
G protein-coupled receptor 44	GPR44	−1.07	2.01	2.28
Chemokine (C-C motif) receptor 3	CCR3	−1.02	1.68	1.56
Th1 cytokines and related genes				
Colony stimulating factor 2	CSF2	−1.19	2.11	3.00
Interleukin 12B	IL12B	−1.54	1.86	2.66
Interleukin 2	IL2	1.25	−1.05	2.49
Inhibin, beta A	INHBA	−1.06	2.06	3.03
Other genes related to human T cells				
Interleukin 6	IL6	−1.19	2.00	4.66
Interleukin 17A	IL17A	−1.19	−1.31	4.47
CD28 molecule	CD28	−1.19	5.03	4.19
Tumor necrosis factor (ligand) superfamily, member 4	TNFSF4	1.92	2.29	3.12
Secreted phosphoprotein 1	SPP1	1.34	2.40	2.13

including *CXCL13*, *CCL7*, *CCL17*, *ILIF8*, *IL9* and *C-reactive protein* (*CRP*) increased with increasing severity of asthma.

Gene expression patterns of children with asthma by using human T cells (Th1-Th2-Th3) related gene PCR array

As shown in Table 4, in moderate and severe asthma, 9 and 17 of the T cell-related genes, respectively, were upregulated (fold change ≥ 2.0 and P-value < 0.05). For several of the T cell-related genes, the fold changes increased with increasing asthma severity, similar to the pattern observed for the inflammatory cytokines and receptors (Table 4). Seven of Th2 cytokines and related genes were expressed at higher levels in severe asthma compared with healthy controls, including *CCL11*, *CCL7*, *IL-3*, *IL-10*, *IL-4*, *IL-5*, and *G protein-coupled receptor 44* (*GPR44*). The expression of several Th1 cytokines and related genes was also increased in severe asthma including *colony stimulating factor 2* (*CSF2*), *IL-12B*, *IL-2*, and *inhibin, beta A* (*INHBA*) (Table 4).

Discussion

In this study, we use '24-hour recall method' to compare the difference of energy intake and energy-adjusted nutrition intake between children with and without asthma. As shown in Table 2, intakes of vitamin C in mild and moderate asthma groups were significantly higher than that in healthy control. Vitamin E intake in mild asthma group, also, was significantly higher than that in healthy control. However, we could not find any significant differences in intake of energy, protein, fat, carbohydrate, vitamin B group, and mineral. Vitamin C and E are both antioxidants that have previously been associated with a reduced prevalence of asthma[19]. For example, vitamin C can suppress the inflammatory response in asthma patients by decreasing reactive oxygen species (ROS) and inhibiting lipid peroxidation[20,21]. Vitamin C also plays key role in the regeneration of vitamin E[22]. Vitamin E intake has been associated with decreased allergic skin sensitization and serum Ig E levels[23]. Similar to vitamin C, vitamin E supplementation also reduced the production of superoxide[24]. Based on these results, we speculate that children with mild asthma have ingested greater amounts of antioxidant-rich foods such as fruits and vegetables as a means to improve their symptoms of asthma.

The inflammatory response in asthma is dominated by T lymphocytes, eosinophils, and master cells[25,26]. A range of cytokines and chemokines secreted by these immune cells are known to play crucial roles in airway inflammation induced by allergen exposure[27]. To investigate the cytokine and chemokine gene expression profiles in the peripheral blood of children

with asthma, we used one PCR array focused on inflammatory cytokines and receptors, and a second PCR array focused on human T cell-related genes (Th1-Th2-Th3). Our inflammatory cytokines and receptor PCR array was showed that mRNA expression of *CXCL13* in severe asthma was the most upregulated among all three asthma groups. CXCL13 is secreted by dendritic cells and its precise role in allergic airway disease is not yet known. The expression of *CXCL13* was increased in a mouse model of allergic airway inflammation and in young patients with asthmatic chronic rhinosinusitis with nasal polyps[15,28]. Moreover, several Th1 cytokine related gene was increased in severe asthma. Especially, INHBA, which is recent studies have also described some Th2 properties for this gene, was increased in severe asthma[29]. Our results are consistent with other studies showing that the expression of INHBA is increased in $CD4^+$ T cells from patients with asthma[12,30]. Finally, in the asthma group, we found that the expression of CCL11, CCL7, IL-13, and secreted phosphoprotein 1 (SPP1, osteopontin) was increased in the PCR arrays of inflammatory cytokines and receptors and human T cell-related genes (Tables 3 and 4). SPP1, which is not previously been as associated with asthma, is known to influence the Th1/Th2 balance and dendritic cell differentiation. Similar to our PCR array results, the expression of SPP1 was increased in the lungs of asthmatic subjects[12,31]. Therefore, we have identified three genes, CXCL13, INHBA and SPP1, that may have crucial roles in childhood asthma but whose likely mechanism of action is uncertain.

The etiology of asthma is complex, involving interacting environmental and genetic factors[32-34]. Diet has been identified as a potential factor in the development of asthma, and may interact at the genetic level[34]. For example, vitamin C can reduce the TNF-α, IL-6, and IL-18 levels in acute inflammation[35], and vitamin E can reduce the production of TNF-α, IL-1β and IL-8 by stimulating leukocytes and reduced levels of IL-6, TNF-α and IL-1β by monocytes[28,36,37]. These studies are consistent with our PCR array results showing that the mild asthma group, which had high intake of vitamins C and E, expressed reduced levels of cytokines compared to the other two asthma groups and the healthy control. For example, we found that expression of IL-10, IL-4, IL-5 and IL-6 in the mild asthma group was downregulated in comparison to healthy controls (Table 4).

In conclusion, we observed the differential expression of cytokine and chemokine genes in children with asthma. In particular, cytokine gene expression was downregulated in the mild asthma group, which had a higher intake of vitamins C and E. Since most asthma begins in childhood, we speculate that regulation of immune genes by dietary factors is important determinant of asthma severity.

Materials & Methods

Subjects

Children with asthma who visited Korea University Anam Hospital Environmental Health Center participated in the study. Healthy children were recruited with the use of mailings and posters. Pediatricians assessed lung function and forced expiratory volume in one second (FEV_1), and determined the diagnosis and severity of asthma according to the American Thoracic Society criteria. For each subject, asthma was classified as mild ($n=18$), moderate ($n=8$), or severe ($n=5$), and their profiles are summarized in Table 1.

This study was approved by the institutional research ethics committee at Korea University Anam Hospital. Children and their parents provided informed written consent.

Dietary survey

Daily dietary intake was recorded using a '24-hour recall method'. The parents of the study subjects were instructed how to record the weights of their meals and to record food intake using standard household measurements (e.g., spoonfuls and cups). Energy intake and energy-adjusted nutrition intake were calculated with a computer-aided dietary analysis system (CAN-Pro. Version 3.0, The Korean Nutrition Society, 2006).

Real-time (RT) PCR array

A total of 2.5 mL of peripheral blood from children ineach group was collected into a PAXgene™ Blood RNA tube (BD, Franklin Lakes, NJ). Total RNA was isolated with the PAXgene™ Blood RNA Kit (Qiagen Inc., Valencia, CA) according to the manufacturer's instructions and was used as a template for synthesis of first-strand complementary DNA (cDNA). The synthesized cDNA was mixed with RT2 SYBR green/ROX qPCR master mix (SABiosciences Corporation, Frederick, MD, USA) and 25 μL of the mix was loaded into each well of the PCR Array.

We used two different RT^2 Profiler™ PCR arrays (Superarray, Gaithersburg, MD, USA): the inflammatory cytokines and receptors RT^2 Profiler™ PCR array and the Human helper T cell (Th1-Th2-Th3)-related genes RT^2 Profiler™ PCR array. Each PCR array includes 84 human genes. The PCR array data were

analyzed using SuperArray PCR Array Data Analysis software (SABiosciences Corporation) with the ΔΔCt method. The ΔCt value for each gene was calculated after normalization with 5 housekeeping genes including *β-2-microglobulin* (*B2M*), *hypoxanthine phosphoribosyltransferase 1* (*HPRT1*), *ribosomal protein L 13a* (*RPL13A*), *GAPDH*, and *β-actin* (*ACTB*). The ΔΔCt value for each gene was calculated as the difference between the ΔCt of the children with asthma and the ΔCt of healthy control. The fold change for each gene was calculated as $2^{-\Delta\Delta Ct}$, and then presented as average fold change for genes.

Statistical analysis

All data were expressed as mean ± standard deviation. After ANOVA, Duncan's multiple range test was performed to determine the significance of the differences in intake of energy and energy-adjusted nutrition. The differences were considered significant at the 5% level.

Acknowledgements This subject is supported by Ministry of Environment as "The Eco-technopia 21 project (2008-09002-0012-0)" from Korea Institute of Environmental Science and Technology (KIEST) and Korea University Anam Hospital Research Center for Environment and Health.

References

1. Aoki, T. *et al*. Expression profiling of genes related to asthma exacerbations. *Clin Exp Allergy* **39**:213-221 (2009).
2. Downs, S. H. *et al*. Continued increase in the prevalence of asthma and atopy. *Arch Dis Child* **84**:20-23 (2002).
3. Akinbami, L. J. & Schoendork, K. C. Trends in childhood asthma: prevalence, health care utilization, and mortality. *Pediatrics* **100**:315-322 (2002).
4. Hijazi, N., Abalkhail, B. & Seaton, A. Diet and childhood asthma in a society in transition: a study in urban and rural Saudi Arabia. *Thorax* **55**:775-779 (2000a).
5. Wickens, K. *et al*. Fast-goods - are they a risk factor for asthma? *Allergy* **60**:1537-1541 (2005).
6. Huang, S. L. *et al*. Dietary factors associated with physician diagnosed asthma and allergic rhinitis in teenagers: analyses of the first Nutrition and Health Survey in Taiwan. *Clin Exp Allergy* **31**:259-264 (2001).
7. Fogarty, A. & Britton, J. The role of diet in the aetiology of asthma. *Clin Exp Allergy* **30**:615-627 (2000).
8. Kim, J. H., Ellwood, P. E. & Asher, M. I. Diet and asthma: looking back, moving forward. *Respir Res* **10**:49-56 (2009).
9. Hatch, G. E. Asthma, inhaled oxidants, and dietary antioxidants. *Am J Clin Nutr* **61(Suppl.)**:625S-630S (1995).
10. Devereux, G. *et al*. Low material vitamin E intake during pregnancy is accociated with asthma in 5-year-old children. *Am J Respir Crit Care Med* **174**:499-507 (2006).
11. Miller, R. L. & Ho, S. M. Environmental epigenetics and asthma: current concepts and call for studies. *Am J Respir Care Med* **177**:657-673 (2008).
12. Hansel, N. N. *et al*. Analysis of $CD4^+$ T-cell gene expression in allergic subjects using two different microarray platforms. *Allergy* **63**:366-369 (2008).
13. Hamid, Q. & Tulic, M. Immunobiology of asthma. *Annu Rev Physiol* **71**:489-507 (2009).
14. Palmqvist, C., Wardlaw, A. J. & Bradding, P. Chemokines and their receptors as potential targets for the treatment of asthma. *Brit J Pharmacol* **151**:725-736 (2007).
15. Lukacs, N. W., Hogaboam, C., Campbell, E. & Kunkel, S. L. Chemokines: function, regulation and alteration of inflammatory responses. *Chem Immunol* **72**: 102 (1999).
16. Zlotnik, A. & Yoshie, O. Chemokines: a new classification system and their role in immunity. *Immunity* **12**:121-127 (2000).
17. Fulkerson, P. C., Zimmermann, N., Hassman, L. M., Finkelman, F. D. & Rothenberg, M. E. Pulmonary chemokine expression is coordinately regulated by STAT1, STAT6, and IFN-gamma. *J Immunol* **173**: 7565-7574 (2004).
18. Ying, S. *et al*. Enhanced expression of eotaxin and CCR3 mRNA and protein in atopic asthma. Association with airway hyperresponsiveness and predominant co-localization of eotaxin mRNA to bronchial epithelial and endothelial cells. *Eur J Immunol* **27**:3507-3516 (1997c).
19. Kalayci, O., Besler, T., Kilinc, K., Sekerel, B. E. & Saraclar, Y. Serum levels of antitoxidant vitamins (alpha tocopherol, beta carotene, and ascorbic acid) in children with bronchial asthma. *Turk J Pediatr* **42**:17-21 (2000).
20. Wood, L. G. & Gibson, P. G. Dietary factors lead to innate immune activation in asthma. *Pharmacol Ther* **123**:37-53 (2009).
21. Patel, B. D. *et al*. Dietary antioxidants and asthma in adults. *Thorax* **61**:388-393 (2006).
22. Chan, A. C. Partners in defense, vitamin E and vitamin C. *Can J Physiol Pharmacol* **71**:725-731 (1993).
23. Zingg, J. M. Vitamin E and mast cells. *Vitam Horm* **76**:393-418 (2007).
24. van Tits, L. J., Demacker, P. N., de Graaf, J., Hak-Lemmers, H. L. & Stalenhoef, A. F. alpha-tocopherol supplementation decreases production of superoxide and cytokines by leukocytes ex vivo in both normolipidemic and hypertriglyceridemic individuals. *Am J Clin Nutr* **71**:458-464 (2000).
25. Davies, D. E., Wicks, J., Powell, R. M., Puddicombe, S. M. & Holgate, S. T. Airway remodeling in asthma: New Insights. *J Allergy Clin Immunol* **111**:215-225 (2003).

26. NIH. *National Heart Lung and Blood Institute NIH guidelines for the diagnosis and management of asthma*. Washington, DC. US Government Printing Office (2002).
27. Singh, V. What is new in the management of childhood asthma? *Indian J Pediat* **75**:845-853 (2008).
28. Devaraj, S. & Jialal, I. Alpha-tocopherol decreases interleukin-1 beta release from activated human monocytes by inhibition of 5-lipoxygenase. *Arterioscler Thromb Vasc Biol* **19**:1125-1133 (1999).
29. Ogawa, K. *et al*. Activin A functions as a Th2 cytokine in the promotion of the alternative activation of marcropharge. *J Immuno* **177**:6787-6794 (2006).
30. Karagiannidis, C. *et al*. Activin A is an acute allergen-responsive cytokine and provides a link to TGF-beta-mediated airway remodeling in asthma. *J Allergy Clin Immuno* **117**:111-118 (2006).
31. Xanthou, G. *et al*. Osteopontin has a crucial role in allergic airway disease through regulation of dendritic cell subsets. *Nat Med* **13**:570-578 (2007).
32. Castro-Giner, F. *et al*. Gene-environment interactions in asthma. *Occup Environ Med* **63**:776-786 (2006).
33. Martinez, F. D. Gene-environment interactions in asthma: with apologies to William of Ockham. *Proc Am Thorac Soc* **4**:26-31 (2007).
34. Kim, J.-H., Philippa, E. E. & Asher, I. M. Diet and asthma: looking back, moving forward. Respiratory Research. *Respir Res* **10**:49 (2009).
35. Du, W. D. *et al*. Therapeutic efficacy of high-dose vitamin C on acute pancreatitis and its potential mechanisms. *World J Gastroenterol* **9**:2565-2569 (2003).
36. Devaraj, S. & Jialal, I. Alpha tocopherol supplementation decreases serum C-reactive protein and monocyte interleukin-6 levels in normal volunteers and type 2 diabetic patients. *Free Radic Biol Med* **29**:790-792 (2000).
37. Devaraj, S. & Jialal, I. Alpha-tocopherol decreases tumor necrosis factor-alpha mRNA and protein from activated human monocytes by inhibition of 5-lipoxygenase. *Free Radic Biol Med* **38**:1212-1220 (2005).