Cellular Defense System Gene Expression Profiling of Human Whole Blood: Opportunities to Predict Health Benefits in Response to Diet$^{1,2}$

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**ABSTRACT**

Diet is a critical factor in the maintenance of human cellular defense systems, immunity, inflammation, redox regulation, metabolism, and DNA repair that ensure optimal health and reduce disease risk. Assessment of dietary modulation of cellular defense systems in humans has been limited due to difficulties in accessing target tissues. Notably, peripheral blood gene expression profiles associated with nonhematologic disease are detectable. Coupled with recent innovations in gene expression technologies, gene expression profiling of human blood to determine predictive markers associated with health status and dietary modulation is now a feasible prospect for nutrition scientists. This review focuses on cellular defense system gene expression profiling of human whole blood and the opportunities this presents, using recent technological advances, to predict health status and benefits conferred by diet.

Introduction

Humans require cellular defense systems to defend against environmental stresses ranging from bacteria, fungi and viruses to those associated with aging, diet and lifestyle. Failure to defend against environmental stresses during our lifetime leads to a number of chronic diseases and resulting morbidities such as cardiovascular disease, hypertension, metabolic syndrome, diabetes, neurodegenerative disorders, and cancers (1–10). Environmental stresses also promote unhealthy aging and increased risk of the aforementioned chronic diseases and associated morbidities (4,11). Control and regulation of our cellular defense systems are thus intimately linked to health status, disease risk, and healthy aging.

Diet is recognized as a critical factor in maintaining cellular defenses. However, mechanistic information on the impact of diet and specific dietary components on maintaining or restoring optimal cellular defense systems and associated health benefits has been problematic. It has been difficult to obtain this information from the necessary human intervention studies due to the inherent problems in accessing target tissues and observed interindividual responses to nutrition. Recent technological advances in gene expression technologies have presented new opportunities to characterize gene expression changes that can be used as predictive markers of health or disease status (12–16). This approach yields information on gene expression changes in target tissues and organs, but it has also been demonstrated that gene expression changes in peripheral blood, as a consequence of nonhematologic disease, can be detected (17–22). After parallel technological advances in gene expression profiling of blood samples (23–29), it is now feasible to interrogate cellular defense system components in human blood samples. Consequently, gene expression signatures predicting health benefits in humans in response to diet are a viable prospect (30,31). This review focuses on modulation of cellular defense system gene expression in response to specific dietary components in human whole blood and developments in blood profiling to predict health status and health benefits of diet.

Cellular defense systems

Humans have 5 key interlinked cellular defense systems: the immune system (32), the inflammatory response (33), redox regulation (1,34), metabolism (2,7,35), and DNA repair (3,4). All 5 cellular defense systems are required to combat diverse stressors and maintain a healthy and functional physiology and reduce disease risk in humans (1–7). These defense systems operate at the molecular level within cells...
and tissues to maintain appropriate functioning of cellular processes and prevent stress-related changes that lead to deteriorating health and increased risk of chronic diseases. Control and regulation of these cellular defense systems are interlinked, often using a common network of gene products and signaling pathways to direct cellular defense and responses to the environment. Diet is a critical factor in the maintenance of these cellular defense systems and one that can be manipulated to ensure health benefits, preventing physiological deterioration and the associated ill health and disease (36–42).

**Strategies for monitoring cellular defense systems in human blood**

It is problematic to assess the impact of diet on these key cellular defense systems in biologically relevant tissues and organs in humans. However, using technological advances, it is now feasible to assess the status of cellular defense systems and responses to dietary factors by gene expression profiling blood samples extracted from the systemic circulation (22,31,38,39,41,43–45). The systemic circulation pervades all cells and tissues. Consequently, changes in the cellular defense systems in cells and tissues can be monitored in blood samples via both the effect on gene transcription induced by secretion of organ-specific factors and the associated changes in the transcriptome associated with blood cell types (31) present in the systemic circulation as a consequence of altered production of blood cells from the bone marrow and thymus. The well-characterized CD markers provide a means of identifying changes in human blood cell types (31) and provide a means of monitoring responses to diet.

Monitoring cellular defense systems in blood to predict health status and responses to diet has presented considerable challenges for researchers. Studies revealed difficulties in reliable detection of RNA transcripts that may be influenced by blood collection and processing methods, sample handling, RNA extraction protocol, and the choice of technological platforms used to assess transcript levels (46–48). Difficulties in obtaining reliable gene expression data proved to be largely due to induction of gene expression due to phlebotomy in whole-blood analysis or the alternative approach involving purification of mononuclear cells from whole blood. Purification methods led to changes in gene transcript levels as a consequence of messenger RNA (mRNA) degradation and gene expression changes during processing of live cells (46–48). Rainen et al. (46) identified RNA degradation and initiation of gene expression in response to whole-blood collection by conventional methods in EDTA tubes followed by an organic extraction of RNA. Spiker et al. (47) reported observations of marked changes in basal gene expression levels in cell populations in response to isolation from whole-blood samples.

Fortunately, technological advances in gene expression profiling of blood extracted from the systemic circulation now present opportunities for interrogation of human whole-blood samples, giving rise to the new field of transcriptional bloodomics (26,30,31). Commercially available systems, such as the PAXgene Blood RNA kit (PreAnalytiX) and the Tempus Blood RNA kit (Applied Biosystems) have revolutionized transcript profiling of human whole-blood samples. These systems permit ease of collection of blood samples directly from donors. The blood RNA profiles are stabilized within the collection tubes using proprietary solutions. Collection and storage at room temperature before frozen storage facilitate the use in human intervention studies because samples can be collected for later RNA extraction. The whole-blood RNA extracted using these commercial systems has proved to be of sufficient yield and quality for gene expression profiling studies (23,25,27–29,49). Extending room temperature storage to the maximum recommended range before freezing has been demonstrated to increase yield and is a useful strategy when sample volumes are limited (25,27). However, it has been noted that differences in the handling of PAXgene blood collections, such as room temperature storage before freezing applied for practical purposes in clinical studies, can contribute to differences in gene expression. However, this was considered to be minimal (0.09% of total variation of measured by Affymetrix arrays) (23). Notably different commercial products for whole-blood collection have also been observed to have an effect on the measurement of differential gene expression of immune activated targets in peripheral blood (29). This highlights the importance of standardization of procedures for collection and processing of blood samples for gene-expression profiling. Application of standardized procedures has permitted satisfactory gene-expression profiles with appropriate pairwise correlations between gene expression profiles using Affymetrix GeneChips (23,25,28) and real-time PCR (27). Further technological advances have permitted increased sensitivity of measuring gene expression in whole-blood samples by depleting abundant globin RNA transcripts (28,49,50). Ryu et al. (50) reported 75% recovery of high-purity mRNA after globin reduction, with an additional 6861 mRNA transcripts detectable in whole blood from volunteers consuming a zinc-depleted diet compared with whole-blood total RNA not subjected to globin reduction. However, it was noted by Liu et al. (49) that processing steps to remove globin transcripts can reduce the quantity and quality of RNA and that transcript profiles may deviate from those of untreated control samples. Novel multiplex gene expression technologies, such as the GenomeLab System (Beckman) now permit assay of multiple targets using nanogram quantities of total RNA (12,51,52). Studies in our lab have established that in combination with the PAXgene system, multiple components of cellular defense pathways in human whole-blood samples can be monitored using the GenomeLab system (52), permitting development of strategies to assess dietary modulation. The technological advances in human blood gene expression profiling discussed previously have led to further exploitation of whole human blood, a readily accessible biological sample, to assess cellular defense system gene expression in response to specific dietary components (discussed later). Plant-based foods, micronutrients, bioactive phytochemicals, high-energy diets, and dietary supplements are notable in this respect and have been subject
to intense investigation to identify the conferred health benefits on cellular defense systems (22,36,39,42,53–61). Differential gene expression in blood has thus been used in recent studies to characterize the impact of a diverse range of dietary factors (22,38–41,44,45,62). The influence of specific dietary components associated with influences on cellular defense systems are discussed further in the following.

**Micronutrients and cellular defense system gene expression in blood**

Various micronutrients have long been associated with health status and links to cellular defense systems, including vitamins, minerals, amino acids, and fatty acids (37,56,63–65). The importance of dietary antioxidant vitamins, such as vitamins C and E, in maintaining redox balance is well-known, but vitamins are also linked to homeostatic regulation of cellular defenses linked to immunity, inflammation, and DNA repair (37,56,64). A vitamin C–rich diet is reported to activate a number of oxidative stress–responsive genes in lymphocytes (antioxidant protein 1 homolog, catalase, cytochrome b-245, copper chaperone for superoxide dismutase, oxidation resistance 1, and oxidative stress responsive 1, peroxiredoxin 4, prostaglandin endoperoxide synthase, cyclooxygenase, and soluble superoxide dismutase 1 (64). Reactive species and oxidative stress in tissues can disrupt the flux of reducing molecules in the blood system, resulting in activation of Nrf2, with antioxidant response elements in the promoter region of redox responsive genes (5). Reports of limited induction of Nrf2 by vitamins (66) imply that there may be additional mechanisms involved in the activation of redox genes in blood cells. Nrf2 induction of antioxidant response element–responsive genes is linked with DNA repair and inflammatory and immune processes (5,67). Vitamin C–rich diets have been linked to induction of Nudix (nucleoside diphosphate–linked moiety X)-type motif (NUDT1) that encodes for 7,8-dihydro-8-oxoguanine triphosphatase, an enzyme preventing nucleotide misincorporation caused by oxidative DNA damage (67).

Dietary vitamin C is also linked to expression of the glutathione peroxidase gene family important in regulating redox balance (67). Likewise, dietary selenium is an important mineral in regulating selenium-containing glutathione peroxidases. Selenium, together with other dietary minerals, zinc, iron, and copper are required for production of many of the enzymes needed for functional cellular defense systems (68). For example, dietary sources of selenium are essential for the maintenance of selenoproteins, which are essential for regulating redox balance, inflammatory responses, and immunity (65). Micronutrients arginine, glutamine, branched chain amino acids, and [n-3] polyunsaturated fatty acids are implicated in the maintenance of healthy immune and inflammatory defenses (69–72). The [n-3] polyunsaturated fatty acids are found in large amounts in fish oils and have been reported to alter peripheral mononuclear blood cell gene expression of signaling pathways controlled by the inflammatory mediator, nuclear transcription factor κB (73). Strategies for enhancing immunity and reducing inflammation using micronutrients have been applied in elderly subjects and cancer patients (37,54). Modulation of immune defense cells and inflammatory mediators appears to be a key factor in immunonutrition (54,69–72,74). Increased arginine and [n-3] fatty acids have been reported to enhance activity of natural killer cells and helper T cells in blood (74). Glutamine has been reported to be an important micronutrient for immune cell functioning of monocytes, lymphocytes, and neutrophils (70).

Notably, studies have also identified potential negative impacts of dietary micronutrients on cellular defense systems (37,54,63,68,75,76). Excess dietary selenium can lead to toxicity (63). Increased mortality from all cancers in smokers with intake of vitamins [multivitamins alone, RR = 1.13 (95% CI: 1.05–1.23) or in combination with vitamin A, C, or E (RR = 1.16 (95% CI: 1.06–1.26)] has been reported (77). Although clinical trials have indicated reduced risk of infection, fewer days on a ventilator, and decreased length of intensive care unit and hospital stay with use of enteral immune-modulating diets, patients with severe sepsis, shock, and organ failure could be disadvantaged by immunonutrition strategies (37). Individuals with systemic inflammatory response syndrome may experience adverse effects on cellular defenses in response to immune-modulating micronutrients such as arginine and unsaturated fatty acids (37). Consequently, an excess of micronutrients can be detrimental, leading to impaired cellular defense and development of disease (63) and increased severity (37,54).

**Phytochemicals and cellular defense system gene expression in blood**

Phytochemicals present in plant-based foods have long been associated with bioactivity in humans, particularly sulfur-containing and phenolic compounds. Sulfur-containing compounds, isothiocyanates, and dithiolethiones derived from cruciferous vegetables, and diallyl sulfides coming from the allium family plants such as garlic and onion are reported to influence cellular defense systems through mechanisms linked to redox balance and DNA stability (40,78). Phenolic compounds in the human diet, such as flavonoids, are among the most studied for their bioactive properties. However, the vast array of phytochemicals in the human diet and extensive metabolism and interindividual responses have led to conflicting reports of the effects of phytochemicals on cellular defenses (79). Recent studies have focused on dietary interventions with phytochemical-rich foods (38,39,43,45,59). A flavonoid-rich diet, including soybeans and derivatives, onions, green tea, and bilberry juice was associated with increased expression of DNA repair and decreased metabolic pathway gene expression in whole blood and a parallel decrease in antimutagenicity measured in urine (38). Flavonoid-mediated detoxification processes have also been linked to the CYP gene family and regulation of the aryl hydrocarbon receptor (80). The aryl hydrocarbon receptor and aryl hydrocarbon receptor nuclear translocator are transcription factors that regulate CYP genes that detoxify mutagenic metabolites that cause...
DNA damage. Antioxidant-rich diets have also been reported to alter expression of DNA repair and aryl hydrocarbon receptor and aryl hydrocarbon receptor nuclear translocator genes in blood (39), concomitant with increased plasma antioxidant markers. Despite the potential for a beneficial effect of increased DNA repair and reduced DNA damage indicated by these studies, other reports have implicated consumption of flavonoids with strand breaks in DNA (81).

Isoflavonoids are a class of flavonoid phenolic compounds that include a number of bioactive compounds, such as phytoestrogens, and are produced by pea or bean family. Soy foods contain considerable amounts of isoflavones and have been linked to altered gene expression of cellular defense genes in peripheral mononuclear blood cells (43). Consumption of isoflavones in soy foods (at least 50 mg/d) was associated with altered blood cell gene expression in metabolic pathways linked to insulin signaling, immune and inflammatory processes (FC receptors, inducible cyclooxygenase, calmodulin, and adducin) (43). However, these studies were performed on a population that mostly was unable to produce the isoflavone metabolite equol from daidzein. Isoflavones are also attributed to cardiovascular protection through targeting of the redox-sensitive Nrf2-Keap1 defense pathway (60). Indeed many phytochemicals have been reported to have antioxidant properties. Phytochemical-rich diets containing soft fruits, food plants of the Brassica family, green tea, herbs, nuts, seeds, and dark chocolate considerably increased bioavailable phenolic compounds and were correlated with increased antioxidant biomarkers in plasma and altered regulation of cellular defense genes involved in DNA repair and metabolic pathways (39). Consumption of the increased phenolic compounds in tomato and green tea extracts has been linked to altered expression of inflammatory genes in peripheral mononuclear blood cells (45).

Deduction of the direct effects of phenolic compounds on regulating cellular defense system genes in human blood is problematic in human intervention studies using phytochemical enriched diets. The food components rich in phytochemicals are also associated with increased consumption of many of the micronutrients that have also been correlated with altered regulation of cellular defenses (38,39,45). Further studies to identify phenolic metabolites present in whole-blood samples, together with in vitro mechanistic studies, are necessary to determine the specific effects on cellular defense gene regulation.

**Macronutrients and cellular defense system gene expression in blood**

Macronutrients have a profound effect on cellular defense systems, giving rise to alterations in the blood transcriptome providing predictive indicators of health status (22,41,43,45). Dietary carbohydrate and protein intake is correlated with changes in the blood leukocyte transcriptome. Differential expression of genes associated with glycolgen metabolism and protein biosynthesis were measured by Affymetrix microarray analysis in response to consumption of either a high-carbohydrate or high-protein breakfast (44). Differential gene expression in response to both the high-carbohydrate or high-protein breakfasts showed similarities in regulation and consisted of 141 genes, including a number of immune-related transcripts (44). It was also noted that there were considerably more differentially expressed genes in leukocytes in response to consumption of the high-protein compared with high-carbohydrate breakfast. It was noted in this study that consumption of the high-protein breakfast induced a number of factors influencing satiety. However, despite inclusion in the microarray chip, the related gene transcripts were not detected on microarray analysis of leukocytes (44). This highlights issues to be considered in blood profiling of cellular defense system gene expression in blood and the choice of sample-processing procedure.

Consumption of energy-dense foods and the resulting obesity, now a worldwide epidemic (82), is characterized by aberrant metabolic regulation. Individuals who are obese exhibit altered metabolism in biologically relevant tissues such as adipose and muscle (41,83,84). These changes are characterized by distinct metabolic changes in peripheral blood. Aberrant blood profiles of hormones, peptides, and lipids measured in obese individuals are linked to the development of diabetes (85), heart disease (86), and cancer (8). Ghosh et al. (22) reported increased expression of genes, such as carbonic anhydrase, ferrochelatase, synuclein, and glycoporphin B, associated with erythrocytes and reticuloocytes, supporting previous observations of higher red blood cell counts in obese individuals (87).

Blood gene expression profiles associated with obesity have identified components of metabolic regulation linked to other cellular defense systems (22,76,88,89). Ghosh et al. (22) reported a decrease in expression of transcripts linked to immune responses. This may be linked to reports of aberrant leptin signaling in obese individuals via activation of leptin receptors expressed by natural killer cells (90). High-energy diets leading to obesity are associated with oxidative stress (89) and inflammation (76,88,91). Low-fat diets (11% of energy from fat compared with control diet of 27% energy from fat) were been linked to cellular defense gene expression changes in blood cells after an 8-week intervention (43). NAMPT showed the greatest fold reduction in response to decreased dietary fat in this study and plays a role in redox reactions in addition to regulating cellular metabolism and possibly insulin secretion (43). A number of transcripts associated with inflammation and immune responses linked to phagocytosis have been reported to be regulated in response to dietary fat (43).

Energy restriction has been reported to restore metabolic health (92). This effect may be a consequence of nutrient-sensing pathway down-regulation of the metabolic rate. Studies in model organisms indicate that energy restriction has an important effect on increasing life span. This is attributed to influencing genes, such as the sirtuins, that are known to be involved in DNA repair (93). Human homologs of genes regulated by energy restriction and associated with increased life span in model systems, such as SIR-2 are known to regulate DNA repair factors (94,95).
Dietary supplements and cellular defense system gene expression in blood

Evidence of beneficial effects of specific dietary components on cellular defenses has led to the production of dietary extracts in an attempt to elicit health benefits. A mixture of 5 bioactive phytochemicals (ashwagandha, Bacopa extract, green tea extract, silymarin, and curcumin) (61), has been reported to reduce plasma markers of oxidative stress (67) and regulate a number of gene markers associated with atherosclerosis, colon cancer, and Alzheimer’s disease (5).

Nutritional supplements, such as cysteine-rich proteins, whey or keratin, and papaya extract (96–98), have been reported to improve antioxidant status and alleviate oxidative stress. Marotta et al. (96) measured considerably increased expression of Nrf2-regulated glutathione peroxidase, superoxide dismutase, and catalase genes in leukocytes. The base excision repair gene 8-oxoguanine glycosylase (hOGG1) was also up-regulated in response to consumption of papaya extract (96). Dietary supplementation with fermented papaya extract (6 g/d for 6 mo) was also shown to down-regulate the inflammatory mediator TNF-α in isolated monocytes (98).

Conclusion

Human blood and the dynamic cell population that it contains may serve as a useful biological indicator to determine predictive signature indicators health status with respect to cellular defense systems and the impact of exposure to diet. Gene predictors linked to cellular defense system signaling pathways and associated with a healthy phenotype are being identified and could potentially be used to predict health status, prepathological changes linked to diet and lifestyle factors, and, crucially, responses to dietary changes to optimize diet and lifestyle for health. Use of blood transcriptomic analysis to determine predictive gene signatures will require further functional and biological validation to generate confidence in prediction of health status. However, this approach is already proving feasible in commercial molecular diagnostics with clinically useful information being obtained from application of assays based on gene expression patterns in blood cells. Assessment of multiple components of cellular defense systems in human blood samples could ultimately permit monitoring and survey of the impact of diet and lifestyle factors to generate evidence for effective translation of research on food, drink, and health.

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Literature Cited


