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Recent Progress in Understanding Host Mucosal Response to Avian Coccidiosis and Development of Alternative Strategies to Mitigate the Use of Antibiotics in Poultry Production

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ABSTRACT As the world population grows and developing countries become more affluent, the global consumption of meat will increase by more than 50% within the next 10 years. Confronting the increased demand for poultry food products are emerging field diseases, increasing regulatory bans of antimicrobial growth promoters, high-density growth conditions, and waste management. Although biotechnology offers solutions to some of these challenges, basic studies are needed to better understand the complex interaction between the intestinal microbiome, host immunity and the environment. This presentation will focus on emerging strategies to enhance gut immunity and to decrease economic losses due to poultry diseases. This presentation will highlight recent developments in coccidiosis research and provide information on host immunity, immunomodulation, and the latest advances in dietary and nutritional approaches against coccidiosis. Such information will magnify our understanding of host-parasite biology, mucosal immunology, and design of future nutritional interventions and vaccination strategies for coccidiosis.

(Key words : coccidiosis, genomics, host immunity, immunomodulation, phytonutrients)

INTRODUCTION

Coccidiosis is a ubiquitous intestinal protozoan infection of poultry seriously impairing the growth and feed utilization of infected animals (Lillehoj et al., 2007; Lillehoj and Lillehoj, 2000). Conventional disease control strategies relied heavily on chemoprophylaxis costing the industry tremendously. Existing vaccines comprise live virulent or attenuated *Eimeria* strains with limited scope of protection against an ever evolving and widespread pathogens. The continual emergence of drug resistant strains of *Eimeria*, coupled with the increasing regulations and bans on the use of anticoccidial drugs in commercial poultry production, urges the need for novel approaches and alternative control strategies. Due to the complexity of the host immunity and the parasite life cycle, comprehensive understanding of host-parasite interactions and protective immune mechanisms becomes necessary for successful prevention and control practices. Recent progress in functional genomics technology would facilitate the identification and characterization of host genes involved in immune responses as well as parasite genes and proteins that eliciting protective host response (Kim et al., 2008a; Kim et al., 2010a).

While natural infection with *Eimeria* spp. induces immunity, vaccination procedures on a commercial scale have shown limited effectiveness and disease control remains largely dependent on routine use of anti-coccidial drugs. Available live vaccines are composed of either virulent or attenuated strains with a major disadvantage consisting of a number of live parasites making them laborious and costly to produce. Although live oocyst vaccines represent a limited but useful alternative to anticoccidial drugs, a vaccine composed of parasite antigens/antigen-encoding genes that elicit specific immunity is eminently preferable. While it would be cost-effective to produce recombinant vaccines (proteins or DNA), the difficulty remains to identify which antigens or genes are responsible for eliciting protective immunity or how these recombinant vaccines should be delivered and presented to the bird's immune system. Also, such subunit vaccines would eliminate the danger of emerging resistant strains encountered with live vaccines but until efficient vaccines become commercially available, the poultry industry is forced to rely upon prophylactic chemotherapy to control the disease. Further, the

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introduction of alternative prevention/treatment measures such as non-chemical feed supplements that effectively enhance productivity and non-specific immunity may help limit the use of anticoccidials. However, the lack of efficient vaccines and the increasing incidence of drug resistant strains and escalating public anxiety over chemical residues in meat and eggs mandate the development of alternative control methods.

INNATE AND ACQUIRED IMMUNE RESPONSES TO *Eimeria*

Following coccidiosis, both circulating and secretory antibodies specific for coccidia parasites are detected in serum, bile and intestine (Lillehoj, 1988; Yun et al., 2000a,b). However, antibody titers in serum and intestine do not correlate with the level of protection after oral infection with coccidia (Lillehoj and Ruff, 1987). Three isotypes of antibodies are recognized in birds, IgM, IgA and IgY, the latter being considered the ortholog of the mammalian IgG (Leslie and Clem, 1969), even though the cDNA encoding the IgY heavy chain shows similarity to mammalian IgE (Parvari et al., 1988). The role of parasite-specific antibodies both in serum and mucosal secretions has been extensively studies in coccidiosis (Lillehoj and Ruff, 1987). In general, antibodies are hallmark of host immune response to *Eimeria* parasites, but are not involved in protection against coccidiosis (Lillehoj and Lillehoj, 2000).

In studies examining the role of T cells in the protection against coccidiosis, the abrogation of T-cell function impaired the development of protective immunity against coccidiosis (Lillehoj and Trout, 1996). Additional evidence for the protective role of T cell came from adoptive transfer studies, in which peripheral blood lymphocytes (PBLs) and splenocytes from *E. maxima*-immune chickens protected syngeneic recipients against a live parasite challenge infection (Rose and Hesketh, 1982). Direct evidence for the presence of *Eimeria*-specific T cells was demonstrated by antigen-specific proliferation of T lymphocytes in *Eimeria*-immune chickens (Lillehoj, 1986; Vervelde et al., 1996). Splenocytes from *E. tenella*-immune chickens inhibited the intracellular development of *E. tenella* in kidney cells *in vitro* (Lillehoj and Choi, 1998). Following primary and secondary infections with *E. acervulina* in chickens, an increased percentage of intraepithelial $\gamma\delta$-T cells were observed in the duodenum, concurrent with a significant enhancement of interleukin (IL)-2 mRNA transcripts (Choi and Lillehoj, 2000). In avian coccidiosis, the selective elimination of $CD8^+$ cells by an anti-$CD8^+$ monoclonal antibody resulted in exacerbation of the disease, as evidenced by increased oocyst shedding after infection with *E. tenella* or *E. acervulina* (Trout and Lillehoj, 1996). Also, significant increase of T cells expressing $CD8^+$ molecules was noted in the intestinal intraepithelial lymphocytes (IELs) following challenge infections with *E. acervulina* (Lillehoj and Bacon, 1991). Bessay and colleagues observed a significant increase in the proportion of $CD4^+$, $CD8^+$ and $\gamma\delta$-TCR cells in duodenal IELs with *E. acervulina* infection, and both $CD4^+$ and $CD8^+$ cecal IELs following *E. tenella* infection (Bessay et al., 1996). The proportion of $CD8^+$ T cells increased in White Leghorn chickens 8 days after a primary infection with *E. tenella* (Breed et al., 1996; Breed et al., 1997a,b), which is concurrent with a marked increase in interferon (IFN)-γ, as well as nitric oxide (NO) production, upon *in vitro* stimulation of PBL by T-cell mitogen and *E. tenella* sporozoite antigen (Breed et al., 1997b). In a recent study, chicken NK-lysin was identified and its functions examined (Hong et al., 2006). The results supported the role of NK-lysin as an important mediator of NK cell activity during avian coccidiosis. Chicken macrophages are involved in different phases of the host immune response to coccidian parasites (Lillehoj et al., 2004). Macrophages pretreated with the culture supernatants of concanavalin A-stimulated splenocytes or T cells exerted cytostatic effects on the growth of *E. tenella* sporozoites (Lillehoj et al., 1989a).

Cytokine and chemokine responses: Extensive experimental evidence supports the notion that immunity mediated by lymphocytes and their secreted products, such as cytokines, mediate antigen-specific protection against challenge infection with *Eimeria* (Lillehoj and Lillehoj, 2000; Lillehoj et al., 2004). In contrast to the plethora of mammalian cytokines, only a few chicken homologs have been described, the major ones including IFN-γ, IL-1, 2, 6 (Schneider et al., 2001), 8, and 15 (Lillehoj et al., 2004; Staeheli et al., 2001). More recently, a series of new chicken cytokines have been described, inclu-

ding IL-17 (Min and Lillehoj, 2002), 18 (Schneider et al., 2000), 16 (Min and Lillehoj, 2004), 12 (Degen et al., 2004), and Th2-type cytokines, such as IL-2, 4, 5 (Koskela et al., 2004), IL-10 (Rothwell et al., 2004), 13 and granulocyte-macrophage colony-stimulatory factor (GM-CSF) (Avery et al., 2004). The chicken gene encoding IFN-γ has been cloned, and its biological function studied (Digby and Lowenthal, 1995; Song et al., 1997). IFN-γ exerts an inhibitory effect against *Eimeria*, and has been shown to enhance protective immunity when used as a vaccine adjuvant against coccidiosis (Choi et al., 1999; Lillehoj and Choi, 1998; Lillehoj et al., 1989a,b). The chicken IL-2 gene has been cloned (Sundick and Gill-Dixon, 1997) and its biological function characterized (Choi and Lillehoj, 2000; Lillehoj et al., 2001). After primary and secondary infections with *E. acervulina*, a significant enhancement of IL-2 mRNA transcripts was observed in the spleen and intestine (Choi and Lillehoj, 2000). The protective effect of IL-2 on vaccination of chickens with recombinant coccidia genes was demonstrated using DNA vaccination model (Lillehoj et al., 2000; Min et al., 2001). Production of chicken IL-6-like activity was detected in serum taken from chickens infected with *E. tenella* during the course of a primary infection (Lynagh et al., 2000). *In vitro* production of IL-1 by macrophages obtained from *Eimeria*-infected chickens was observed during, and immediately following, infection with *E. maxima* or *E. tenella* (Byrnes et al., 1993). Chemokines are important mediators of cell migration during inflammation and in normal leukocyte trafficking. IL-8 and K60 are CXC chemokines (Kaiser et al., 1999; Sick et al., 2000) and K203 is a CC chemokine recently cloned from chickens (Sick et al., 2000). Recent evidence indicated that IL-10 is produced during coccidiosis (Rothwell et al., 2004), but its role in disease pathogenesis has not been fully studied.

GENE EXPRESSION ANALYSIS TO IDENTIFY HOST IMMUNE PATHWAYS ASSOCIATED WITH COCCIDIOSIS

In chickens, a limited number of low- and high-density cDNA microarrays have been developed (Morgan et al., 2001; Min et al., 2003; Neiman et al., 2003; Cogburn et al., 2004; Bliss et al., 2005). In addition, a consortium of research groups has developed a comprehensive 13,000 element chicken cDNA microarray (Burnside et al., 2005) and several whole chicken genome oligonucleotide arrays (Affymetrix Corp., Agilent Tech. etc.) are commercially available.

Two tissue specific chicken cDNA microarrays with about 5,000 and 10,000 preselected EST from macrophages (Bliss et al., 2005) and intestine IEL (Min et al., 2005), respectively, have been used to investigate local gene expression profiles and host innate and adaptive immune responses of broiler chickens infected with *Eimeria*. The avian macrophage microarray (AMM) has been applied to different avian cell or tissue types as well as a variety of pathogens, and both have proven to be useful tools to identify global gene expression changes. For example, changes in expression of genes involved in innate immunity have been observed *in vivo* from experimentally infected chickens and *in vitro* using peripheral blood monocytes, heterophils, nonadherent blood lymphocytes, and multiple avian macrophage cell lines. In addition, innate immune responses have been elucidated following stimulation with *Salmonella*, *Escherichia coli*, *Mycoplasma*, influenza viruses, *Eimeria*, bacterial cellular components (LPS), and plant phytochemical immune modulators using these arrays. Use of the AMM has led to better understanding of the innate immune response mediated by avian macrophages in response to 3 antigenically distinct species of *Eimeria*, *E. acervulina*, *E. maxima*, and *E. tenella* (Dalloul et al., 2007). Using AMM, a set of core response elements was identified comprising 25 genes, including many immune-related genes such as the proinflammatory cytokine IL-1β, the chemokines ah221 and MIP-1β, and osteopontin, whereas 60 to 67 elements were uniquely induced or repressed by the individual species. Such differential responses may be attributed to the species-specific immunity induced by different *Eimeria* species, and a deeper look into the functional aspects of these elements may lead to the elucidation of the pathogenicity, immunogenicity, or both, of each species and better design of a coccidiosis vaccine. *E. acervulina*, *E. maxima*, and *E. tenella* are the most common coccidia encountered in the field, and each infects a unique site in the chicken intestine. Infections, when not deadly, induce protective immunity against subsequent challenges; however, such immunity re-

mains confined to homologous species with no cross-species protection (Lillehoj et al., 2004). Among the 3, *E. maxima* infection is characterized by high immunogenicity, in which priming infection with few oocysts induces full protective immunity to subsequent homologous challenge. Conversely, far more *E. acervulina* and *E. tenella* oocysts are required to induce comparable protective immunity. For these reasons, identification of the early host responses at the gene transcription level provides a molecular immune profile of the events that occur during and immediately following infection with *Eimeria*.

We first previously described a 400 element IEL cDNA microarray that was used to analyze the levels of chicken cytokine mRNAs during *E. acervulina* or *E. maxima* infections (Min et al., 2003). Subsequently, we developed a 4909 array containing genetic elements of avian macrophages that was applied to profile the transcriptional responses during *E. acervulina*, *E. maxima*, or *E. tenella* infections (Dalloul et al., 2007). Most recently, a second-generation 9.6K intestinal IEL cDNA microarray (AVIELA) was constructed with the probes selected from IEL cDNA library of *Eimeria*-infected chickens and the clones related with immune responses and used to study chicken gene expression during experimental coccidiosis (Kim et al., 2008a). Following primary inoculation with *E. maxima*, the expression levels of 74 genes were significantly altered more than two-fold over the 3-day infection period (51 up-regulated, 23 down-regulated). Following secondary infection, the expression levels of 308 genes were significantly altered (62 up-regulated, 246 down-regulated). Pathway gene analysis indicated that many of the modulated genes were related to apoptosis, JAK/STAT, MAPK, interleukin, and TLR signaling pathways, and involving innate and adaptive immune responses. Gene Ontology analysis showed that primary infection significantly modulated the levels of mRNAs for genes involved in the metabolism of lipids and carbohydrates as well as those for innate immune-related genes. By contrast, secondary infection increased the levels of transcripts encoded by genes related to humoral immunity and reduced the levels of transcripts for the innate immune-related genes (Kim et al., 2010a). AVIELA was successfully used to compare the gene expression profiles of two B-complex disparate, genetically inbred Fayoumi chicken lines that differ in susceptibility to *E. maxima* (Kim et al., 2008b). Functional analysis using gene ontology categorized the genes exhibiting the different expression patterns between 2 chicken lines into several gene ontology terms including immunity and defense. The transcriptional profiles showed that more gene expression changes occurred with *E. maxima* infection in the M15.2 than the M5.1 line and the most gene expression differences between the 2 chicken lines were exhibited at 4 and 5 days after *E. maxima* infection.

NUTRITIONAL MITIGATION OF INFECTIOUS DISEASES

There is an increasing interest for developing an alternative control strategy against many infectious diseases of livestock and poultry due to much publicized concerns over the use of drugs for promoting animal health. One promising new avenue to achieve this goal is the use of natural foods and herbal products to enhance host defense against microbial infections and tumors. This approach is based on many scientific data demonstrating the immunomodulatory effects of natural and herbal products in many animal species as well as humans. For many mucosal diseases of poultry, including avian coccidiosis, a new paradigm is needed to develop a safe and effective disease control strategy as many current approaches are not adequate to effectively control newly emerging and re-emerging diseases. Avian coccidiosis is a major parasitic disease of substantial economic significance, estimated to cost the poultry industry greater than $ 3.2 billion in annual losses in the U.S. alone. Historically, the poultry industry has relied upon prophylactic medication to limit the deleterious effects of this disease. However, the increasing governmental bans on in-feed medications and rising consumer concerns about drug residues in the food supply may eventually force the industry to eliminate this practice and develop alternative coccidiosis control strategies.

A growing body of scientific evidence demonstrates the health-promoting effects of plant-derived phytochemicals, chemical compounds derived from plants or fruits. "Phytonutrients" refers to phytochemicals or compounds from edible plants that have been used as health-promoting agents by many cultures for several millennia. In 400 B.C.E., Hippocrates pre-

scribed willow tree leaves (containing salicylic acid) to abate fever. There is abundant evidence from epidemiological studies that phytochemicals can significantly reduce the risk of cancer and may reduce high blood pressure, pain, and asthma. The most popularly used drug for cancer chemotherapy worldwide is Taxol (paclitaxel), a phytochemical initially extracted and purified from the Pacific Yew tree. Taxol possesses antiviral, anti-bacterial, and anti-cancer properties. Many other phytochemicals with potent medicinal properties are currently in clinical trials for treatment of a variety of diseases. Lycopene, for example, from tomatoes is in clinical trials for cardiovascular diseases and prostate cancer. Its beneficial effects may be due to its anti-oxidant and anti-inflammatory effects. While numerous studies have showed disease prevention or immune enhancing effects resulting from oral feeding of plants, only a few reports have examined the specific effects of plant-derived phytochemicals on gut defenses. The intestinal mucosal system plays a central role in the exclusion and elimination of harmful dietary substances in humans and animals. Part of the intrinsic gut defense mechanisms are mediated by the lymphoid system and the intestine contains a relative large component of lymphatic tissues.

Recent studies from our laboratory provided clear evidence that dietary supplements of natural phytochemicals activate innate immunity in poultry and, in particular, enhance protective immune responses against avian coccidiosis. Phytochemicals are plant- or fruit-derived chemical compounds possessing health benefits including promoting tumor killing and increased resistance to infectious diseases caused by bacteria, virus and parasites. However, very limited information is available on the mode of action of most of health-promoting plant phytochemicals. Therefore, in order to obtain a basic understanding of how dietary supplements, such as plant and fruit extracts, exert immunostimulatory effects in poultry, we carried out *in vitro* and *in vivo* feeding trials using an intestinal protozoan disease model, avian coccidiosis. In various *in vitro* studies, culture of chicken spleen lymphocytes with crude extracts from milk thistle, turmeric, shiitake and reishi mushrooms, persimmon, tomato, safflower leaf, plum fruit, and cinnamaldehyde induced significantly higher cell proliferation compared with the untreated control cells. Stimulation of chicken macrophages with crude extracts of milk thistle, shiitake and reishi mushrooms, persimmon, raspberry, safflower leaf, plum fruit, and cinnamaldehyde resulted in robust nitric oxide production to the levels that were similar with those induced by recombinant chicken IFN-γ. Most of phytochemical extracts and cinnamaldehyde inhibited the growth of chicken tumor cells *in vitro*. The levels of mRNAs encoding IL-1β, IL-6, IL-12, IL-18, and tumor necrosis factor superfamily member 15 (TNFSF15) were enhanced in macrophages that were treated with extracts of turmeric or shiitake mushroom compared with the untreated control (Lee et al., 2007, 2008a, 2009a, b, 2010a, 2011a,b). Cinnamaldehyde also directly reduced the viability of *Eimeria tenella* parasites at 10 and 100 μg/ml (P<0.05 and P<0.001, respectively), compared with media controls (Lee et al., 2011a). The effects of plant extracts on enhancing various *in vitro* parameters of protective immunity have been positively correlated with their ability to protect against microbial infections (Lee et al., 2007, 2008a,b, 2009b,c, 2010a,b, 2011 a,b). In several *in vivo* trials, feeding of broiler chickens with diets supplemented with extracts of mushroom, safflower, plum, and cinnamaldehyde consistently enhanced innate immunity and provided enhanced protection against live oral parasite challenge infections. For example, mushroom extracts and cinnamaldehyde significantly protected chickens against weight loss characteristically seen during coccidiosis and promoted parasite killing as indicated by reduced fecal oocyst shedding. Dietary supplementation of young broiler chickens infected with *Eimeria acervulina* using plum, safflower leaf extracts, *curcuma*, *capsicum,* and cinnamaldehyde increased body weight gain, reduced fecal oocyst shedding, and increased cell-mediated immunity as measured by transcriptional changes in key cytokines such as IL-1β, IL-6, IL-15, and IFN-γ (Lee et al., 2008b, 2009c, 2010b, 2011a).

Furthermore, combination of two phytonutrient mixtures, VAC (carvacrol, cinnamaldehyde, and capsicum oleoresin), and MC (capsicum oleoresin and turmeric oleoresin), were evaluated for their effects on chicken immune responses following immunization with a recombinant *Eimeria* profilin protein. Following immunization and infection, chickens fed the VAC- or MC-supplemented diets showed increased body weights, greater profilin antibody levels, and/or greater lymphocyte proliferation compared with non-supplemented con-

trols. Immunized chickens fed the MC-supplemented diet exhibited increased MHC class II^+, $CD4^+$, $CD8^+$, $TCR1^+$, or $TCR2^+$ T cells compared with nonsupplemented controls while chickens on the VAC-containing diet displayed an increase in $K1^+$ macrophages. Finally, the dietary supplementation with VAC or MC alters immune parameters following recombinant protein vaccination and shows vaccine-stimulated immunity against avian coccidiosis (Lee et al., 2011b).

NUTRIGENOMICS TO IDENTIFY IMMUNE PATHWAYS INFLUENCED BY DIETARY IMMUNOMODULATION

Detailed molecular and cellular changes associated with dietary feeding of young broiler chickens with three different phytonutrients (carvacrol, cinnamaldehyde, and capsicum oleoresin) were investigated using high-throughput gene expression analysis. Investigation of genome-wide differential gene expression of transcriptional changes in the intestine between birds fed diets supplemented with phytonutrients and control diet-fed chickens revealed significant molecular and cellular changes affected by dietary feeding with phytonutrients. The total number of IEL genes which were significantly altered (> 2.0 folds) by three different phytonutrients (carvacrol, cinnamaldehyde, and capsicum oleoresin) were 74, 62, and 254, respectively (Kim et al., 2010b). Pathway and gene network analysis using IPA software showed that capsicum oleoresin and cinnamaldehyde significantly modified the pathways related with carbohydrate metabolism such as citrate cycle (*P* value: 1.95×10^{-4}, and 8.91×10^{-4}, respectively), and glyoxylate and dicarboxylate metabolism (*P* value: 2.14×10^{-2}, and 1.82×10^{-2}, respectively). The pathway for glycolysis/ gluconeogenesis was induced by capsicum oleoresin (*P* value: 4.07×10^{-2}). However, in lipid metabolism, only carvacrol treatment showed statistically significant changes associated with androgen and estrogen metabolism (*P* value: 9.55×10^{-3}) and linoleic acid metabolism (*P* value: 4.79×10^{-2}) pathways. IPA network analysis revealed that 7, 9, and 17 biologically relevant networks were associated with cinnamaldehyde, carvacrol, and capsicum oleoresin groups, respectively. Among the networks, three most reliable ones from each treatment were analyzed and top functions representing high-level functions from the functional analysis of a network were identified. The first network from the treatment group of cinnamaldehyde includes 18 focus genes related to the functions of antigen presentation, humoral immune response, and inflammatory disease. To further validate biological functions of carvacrol, cinnamaldehyde, and capsicum oleoresin in a poultry infectious disease model, broiler birds fed with standard diet supplemented with the plant extract mix designated as XT6930 (5 mg/kg of carvacrol, 3 mg/kg of cinnamaldehyde, and 2 mg/kg of capsicum) were orally challenged with *E. acervulina*. Feeding with XT6930 significantly enhanced body weight gains and reduced gut lesions of *E. acervulina*-infected chickens (Lee et al., 2010b). Furthermore, broiler chickens which were continuously fed with a standard diet supplemented with carvacrol, curcuma and capsicum from hatch showed significantly reduced gut lesion and lower pro-inflammatory cytokine gene expression post challenge infection with *E. acervulina* compared to the controls fed only the standard diet.

CONCLUSIONS

In view of increasing consumers' concerns about drug residues in the food chain, the poultry industry will eventually discover alternative methods to control economically important avian disease. In this regard, dietary immunomodulation of gut immunity using plant-derived phytochemicals is a novel and promising control strategy for avian coccidiosis, and other potential phytonutrients should be explored for other diseases and other livestock species. Furthermore, the underlying immune mechanisms involved in plant phytochemical-mediated immune enhancement of immunity should be investigated in order to maximize its effect and develop a rational program of disease control. Recent studies using plant- derived phytonutrients demonstrate that dietary immunomodulation mediated by the combination of carvacrol, cinnamaldehyde and capsicum oleoresin enhanced coccidiosis resistance. Microarray analysis indicated that these phytonutrients mediated genetic changes associated with metabolism and cell- mediated immunity which were reflected on enhanced protective immune response to avian coccidiosis. These results provide clear evidence for a synergistic effect of the different phytonutrients in promoting local protective immunity against experimental

avian coccidiosis.

The results of these studies provided clear evidence to support the notion that plant-derived phytochemicals possess immune enhancing properties in chickens and this strategy opens a new avenue to develop effective strategies for disease control in poultry without using artificial chemicals. However, very limited information is available on the mode of action of most of health-promoting plant phytochemicals. In an effort to understand underlying molecular mechanisms mediated by phytonutrients, we have applied high-throughput genomics technology. Recent studies in clinical medicine demonstrated that nutrigenomics is providing novel information concerning the molecular and genetic mechanisms of dietary modulation of host immunity, physiology and metabolism. This emerging field of functional nutritional genomics, called nutrigenomics, has created unprecedented opportunities for increasing our understanding of how nutrients modulate gene and protein expression to influence cellular metabolism. Large-scale gene expression profiles provide enormous information on the interactions between nutritional stimuli and host genes. The microarray technique is an ideal tool for gene expression profiling of a large number of genes in a single assay using tissue-specific array platforms.

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