Special Report

Symposium on Diet, Nutrition and Immunity

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The symposium on Diet, Nutrition and Immunity held in Singapore on April, 2008, reviewed the current scientific information on the development of the immune system particularly in infancy and the role of diet, exercise and aging on immunocompetence, together with the molecular processes involved. The importance of specific nutrients particularly zinc and iron, vitamins D and E and long-chain polyunsaturated fatty acids on enhancing immunity, as well as the significance of maintaining a healthy microbiota in the gut leading to the concept of pro- and pre-biotics was discussed. Of interest was the presentation of studies on some common Asian foods such as soybeans, wolfberry, and mushroom that may have application in the prevention and treatment of disease involving inflammatory responses.

Key Words: Diet, immune system, micro- and macronutrients, pro- and pre-biotics, Asian foods

SUMMARY

Symposium on diet, nutrition and immunity
The 2-day symposium on Diet, Nutrition and Immunity reviewed the current scientific information on the role of nutrients and bioactive components in the development of the immune system and the effects of stress, exercise and aging on immunocompetence. An important feature of the symposium was the discussion on the potential contribution of Asian functional foods in disease prevention and treatment. The symposium, sponsored by the International Life Sciences Institute Southeast Asia Region (ILSI SEAR), was held in Holiday Inn Atrium Hotel in Singapore on April 16-17, 2008.

Dr Howard Delaney, President of ILSI SEAR, welcomed the speakers and participants from the Asia Pacific region, as well as from North America and Europe.

The first session dealt with an overview of the immune system and the role of nutrition in its development. The first speaker was Prof Chan Son-Ha of the National University of Singapore and the Yong Loo Lin School of Medicine, Singapore. Prof Chan first discussed the mechanism by which cell mediated and humoral mediated immunity develop, resulting in immune responses that provide defense from external and internal influences. When one or more component of the immune system is defective, immunodeficiency develops such as in malnutrition and old age, chemotherapy and irradiation. Inadequate immune response is also found in the very young, in chronic illnesses such as tuberculosis and diabetes, and physical stress as in acute exercise and prolonged training. On the other hand, immunity can also be harmful as in the case of hypersensitivity to certain allergens or when the body is unable to distinguish self from non-self, leading to autoimmunity, disease, citing thyrotoxicosis, myasthenia gravis, and rheumatoid arthritis as examples. Prof Chan concluded that immune response could be augmented by nutritional factors, but randomized control trials (RCT) on human subjects are needed to confirm these observations. The next speaker in the session was Dr Irmeli Auli Pentilla of Women’s and Children’s Health Research Institute, Australia, who discussed immune development during infancy, particularly the effect of breast milk and weaning on programming the mucosal immune response. Dr Pentilla stressed the importance of proper balance between Th1 and Th2 cytokines in infants, which is required for immunological protection against external pathogens. While breastfeeding has been associated with a protective effect against the development of allergy, the duration of exclusive breastfeeding and the timing of the start of weaning are important factors which need to be considered. In their work with healthy and allergy-prone rat pups fed formula supplemented with TGF-β2, both pups regulated their immune response and this immune regulation persisted after weaning when TGF-β was no longer present in the diet. Dr Pentilla also cited other studies demonstrating oral tolerance in infants and animal models. The PROBIT study in Belarus found that prolonged and exclusive breastfeeding did not protect against eczema, hay fever and asthma. Further, it appears that oral tolerance may be induced with increasing amounts of cow’s milk and other food antigens. Dr Pentilla concluded that further studies are warranted on breastfeeding and immunity, and when to introduce food antigens into the diet.

Dr Pedro Gutiérrez-Castellón, Research Director of the National Pediatric Institute, Mexico, next discussed the importance of nutritional support for the immune system of the new born baby and infant. Starting from innate immunity of the fetus during intrauterine life, passive

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immunity is provided for the newborn by maternal IgG antibodies which are transported transtemporally during the last trimester of pregnancy, and by IgA antibodies in breast milk. In addition, breast milk contains numerous components that protect the infant against infections. Following the composition of breast milk, infant formulas have attempted to incorporate components known to be essential for the infant’s immune system, based on scientific studies. Dr Gutiérrez-Castrellón briefly discussed the results of their systematic review of RCTs comparing the effect on infant immunity of ribonucleotide-supplemented infant formulas (RSIF) with formulas without nucleotide or with breast milk. Their systematic review and meta-analysis showed a generally positive effect of RSIFs on infant immunity in terms of antibody response to immunization and fewer episodes of diarrhea. He concluded that while much remains to be learned about the functional expression and age-dependent maturation of innate immune molecules at birth, nutritional support is important to sustain the infant’s immune system through breast feeding and infant formula supplemented with specific ingredients such as nucleotides.

At the second session of the conference which dealt with micronutrients and immunity, Prof Sunil Sazawal of John Hopkins School of Public Health, USA, described their study on the effect of micronutrient supplementation on immune response and infectious morbidity among 1-3 yr old children in a peri-urban population of New Delhi, India. The study evaluated the effect of consumption of milk fortified with zinc and iron, together with vitamins C, E and A, selenium and copper for one year. The study showed the positive effect of the intervention on immunity as shown by significant reduction in the incidence and prevalence of febrile illness, diarrhea, acute lower respiratory tract infection, and measles. The fortification also improved growth and iron levels, and reduced the proportion of children with iron deficiency and anemia.

Dr Sazawal concluded that fortification of milk with micronutrients is an attractive approach to delivery of micronutrients, especially iron and zinc. Prof Sazawal was followed by Dr Adrian Martineau, a Clinical Research Fellow at Barts and the London School of Medicine, UK, who discussed the immunomodulatory actions of vitamin D. Dr Martineau reviewed studies that indicate that vitamin D deficiency is associated with autoimmune disease such as type I diabetes, rheumatoid arthritis and multiple sclerosis, possibly through its effect on T\textsubscript{H}1-mediated dencentritic cell-macrophage loop. Recently, attention has been focused on the ability of calcitriol to up-regulate expression of genes coding for antimicrobial peptide possessing broad-spectrum anti-bacterial and anti-viral activity. He described their in vitro studies on the mechanism by which 1,25(OH)\textsubscript{2}D\textsubscript{3} increases the ability of mononuclear cells (PBMC) to resist mycobacterium tuberculosis by up-regulating the expression of cathelicidin, an antimicrobial peptide that is known to disrupt bacterial cell membrane and induce neutrophil chemotaxis. He also described their randomized controlled trial among some 200 adult tuberculosis contacts which showed the effectiveness of a single dose of vitamin D in enhancing immunity to mycobacteria. Dr Martineau concluded that vitamin D supplementation could have potential application in the prevention of both T\textsubscript{H}1-mediated autoimmune disease and infectious disease, and as an adjunct to therapy for these conditions.

The third session of the conference dealt with the role of diet on the effect of stress, exercise and ageing in immunity. The first speaker was Prof Dayong Wu of the Nutritional Immunology Laboratory at Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University, USA, who discussed the role of vitamin E in improving immune response in the elderly. Studies have shown that vitamin E modulates immune cell functions and that vitamin E intake above the RDA level may provide beneficial effect on immune system. Their double blind placebo controlled trial among the elderly showed positive effect of supplementation with 200 IU/d of vitamin E on the incidence of upper respiratory infection. Recent investigations into the cellular and molecular mechanisms on the effect of aging on immune function have revealed that it involves multiple stages from early receptor activation to ultimate clonal expansion. One consequence of these changes is the down-regulation of IL-2 synthesis and its signaling activity, leading to hindered T cell division cycle and expansion. In addition, the work of Prof. Wu has shown that age-related increase in PGE\textsubscript{2} production is due to increased COX-2 mRNA and protein levels leading to higher COX enzyme activity, and that the ceramide-induced up-regulation of NFkB activation in old macrophage maybe a central mechanism. Moreover, their recent findings indicate that similar to macrophages, adipocytes from old mice have a higher expression of COX-2 as well as inflammatory cytokines IL-1, IL-6 and TNF. Prof Wu concludes that adipose tissue may be an important tissue contributing to the inflammation associated with aging and may have significant implications for inflammatory diseases developed with aging. The next speaker in the session was Dr Daniel Raederstorff of DSM Nutritional Products, Switzerland, who discussed the role of omega-3 polyunsaturated fatty acids (LC-PUFA) on immune and inflammatory response. He first summarized placebo controlled studies using omega-3 LC-PUFA on inflammatory conditions and observed that evidence of the clinical efficacy of omega-3 LC-PUFA is strong in rheumatoid disease but is weaker in inflammatory bowel disease. Dr Raederstorff mentioned the potential impact of omega-3 LC-PUFA on chronic diseases with inflammatory response, such as obesity, diabetes, cardiovascular disease and neurodegenerative disease of ageing. He discussed the role of omega-3 LC-PUFA on immune regulation, citing studies that demonstrated its protective effect against atopic asthma in adults and children. He described their study on maternal fish oil supplementation in pregnancy and its effect on immune response of infants at high risk of atopy. Their results showed that infants of mothers supplemented with omega-3 PUFA were less likely to be sensitized to allergens and to have severe atopic dermatitis at 1 yr of age. Finally, Dr Raederstorff cited recent evidence suggesting that in early life, omega-3 LC-PUFA accelerates the maturation of the immune system and promotes T\textsubscript{H}1 response, while in later life, it decreases the T\textsubscript{H}1 response to reduce the risk of chronic inflammatory disease. The next speaker in the session was Prof Pedro Gutiérrez-
against allergic disease, such as interacting with mucosal
sensible mechanisms by which probiotics might protect
Royal Children’s Hospital, Melbourne, Australia, who
function. The first speaker was
and bioactive food components that enhance immune
skeletal muscle brought about by exercise.
intracellular mediators of stress and inflammation in
inflammatory action of phytochemicals needs further
protein requires further analysis, and the reported anti-
ment of atopic eczema in early life. However, postnatal
administration of L. acidophilus strain (LAVRI A1) has
failed to protect against atopic eczema. Further studies are
underway to confirm the beneficial effects of probiotics
in the prevention of eczema. Studies studying the use of
probiotics in the treatment of allergic disease such as in-
fant eczema, allergic rhinitis and asthma have been con-
flicting. Prof. Tang concluded that probiotics are probably
more effective when used early in life particularly for the
prevention rather than treatment of allergic disease. How-
ever, there is currently insufficient evidence to support
the application of probiotics for the prevention and treat-
ment of allergic disease. The next speaker in the session
was Prof Lee Yuan Kun of Yong Loo Lin School of
Medicine, National University of Singapore, who spoke
on lactic acid bacteria strains that modulate inflammation
responses. Of the 27 strains of intestinal lactobacilli iso-
ated from infants that they tested, only 4 strains showed
immunomodulating properties, suggesting that the well-
being of infants could be determined by the strains of
lactobacilli that harbor their intestinal tract. Some of these
genes that are modulated are up-regulated, while others
are down-regulated. Ribonucleic acid and protein assay
showed that c-Jun N-terminal kinases (JNK) nd p-38 are
involved in the anti-inflammatory effects of lactic acid
bacteria. Prof. Lee briefly discussed the signaling path-
ways in regulating inflammation responses in the human
intestine. In their in vitro experiments, they found that
lactobacilli inhibit cytokine receptors on the cell surface
and further inhibit JNK and p38 by suppressing their pro-
tein phosphorylation and up-regulating DSP1. Studies
such as these can be useful in the development of effec-
tive therapies for inflammatory diseases such as allergy,
irritable bowel syndrome, and cancer. The next speaker in
the session was Dr Patricia Lynn Conway from the
Center for Marine Biofouling and Bioinnovation of the
University of New Southwales, Australia. Prof. Conway
discussed the various factors influencing their composi-
tion including feeding practices, mode of delivery, and
environmental factors. Thus, it has been shown that Cae-
sarian delivery delays the colonization with bifidobacteria
and is linked to increased risk of allergy, asthma and in-
fec tious diseases, while early exposure to farm animals
reduces the prevalence of allergic conditions in young
children. It is now clear that breast milk harbors benefi-
cial bacteria, mostly bifidobacteria, which is linked to
atopy and allergy of the mother, and should be considered
an important factor in the development of healthy infant-
tile microbiota. This is akin to the use of probiotics in
infants to favor the establishment of a healthy microflora
in the intestine. Studies have shown that probiotics in
infants is helpful in the prevention of diarrheal disease,
Improves innate and adaptive immunity, reduces the risk

Casterellón who discussed immunonutrition in critical
illness. He observed that so far numerous studies includ-
ing several meta-analyses have failed to show consist-
tently positive effect of immunonutrition on mortality of
critically ill patients. On the one hand, studies have
shown that arginine supplementation may be associated
with harm in septic critically ill patients, and is thus not
recommended for such patients. On the other hand,
glutamine supplementation has been shown to have a
positive effect on mortality, infectious complications and
length of hospital stay. Likewise antioxidants such as
selenium may also have an effect on mortality. Studies on
the effect of n-3 fatty acids have shown mixed results. In
the face of these conflicting results, Prof Gutiérrez-
Casterellón hypothesized that a fixed mixed cocktail ap-
proach to immunonutrition does not suit every patient, but
that the administration of nutrients in supranormal
amounts in patients tested individually would have
"pharmacologic" effects on the inflammatory response to
critical illness and can improve clinical outcomes. Profes-
sor Gutiérrez-Casterellón recommended that future stud-
ies on this pharmaconutrition paradigm should be con-
ducted, analogous to the assessment of any pharma-
cologic agent. The last speaker in the session was Prof
David Cameron-Smith of the School of Exercise and
Nutritional Sciences, Deakin University, Victoria, Aus-
tralia. Prof Cameron Smith reviewed the recent studies on
the role of nutrition in regulating the immunological re-
sponses to exercise. It is now known that intense muscu-
lar activity activates a variety of stress-sensitive kinases
whose pathways activate gene transcription resulting in a
rapid adaptive cellular response. In addition, these stress-
responsive pathways activate genes coding for secreted
cytokines and chemokines (myokines) which locally
promotes macrophage chemotaxis and activation, satellite
cell activation, and angiogenesis. Moreover, stress re-
ponse genes have systemic influence on whole body
immunity and inflammation. Since reactive oxygen spe-
cies (ROS) are known to affect these stress sensitive
kinases, studies have evaluated the effect of a variety of
anti-oxidants on markers of muscle trauma and inflamma-
tion. However, most of these studies have shown only
modest and variable impact. Similarly, fish oil supple-
mentation appears to have limited impact on plasma cy-
токines. Of current interest is the impact of polyphenols
on the immunologic response to exercise, although at the
present time available data is limited. The impact of pro-
tein requires further analysis, and the reported anti-
inflammatory action of phytochemicals needs further
studies as well. Greater attention should be given to the
intracellular mediators of stress and inflammation in
skeletal muscle brought about by exercise.

The fourth session of the conference dealt with foods
and bioactive food components that enhance immune
function. The first speaker was Prof Mimi Tang from the
Royal Children’s Hospital, Melbourne, Australia, who
discussed the role of probiotics for the treatment and pre-
vention of allergic disease. Prof. Tang reviewed the pos-
sible mechanisms by which probiotics might protect
against allergic disease, such as interacting with mucosal
immune system, enhancing commensal bacteria coloniza-
tion while inhibiting the growth of pathogenic bacteria, or
suppressing inflammatory immune responses. Probiotics
may induce dendritic cells that promote tolerogenic or
suppressor immune responses, but again the effects on
dendritic cells are species and strain dependent. On the
matter of probiotics for the prevention of allergic disease,
administration of selective strains of probiotic bacteria to
mothers in the last weeks of pregnancy and to the off-
spring in the first 6 to 12 months has been consistently
associated with a protective effect against the develop-
ment of atopic eczema in early life. However, postnatal
administration of L. acidophilus strain (LAVRI A1) has
failed to protect against atopic eczema. Further studies are
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to infantile atopic eczema, and may be useful in the prevention of allergic disease. The use of probiotics in the treatment of pediatric atopic dermatitis, however, is not conclusive. Prof. Conway concluded that the use of strain-specific probiotics could be a safe, scientifically and clinically proven strategy that could provide long term health benefits, especially for infants and also for the elderly. The third speaker in the session was Dr Johan Garssen, Head of the Immunology Section at Numico Research, Wageningen, The Netherlands, who spoke on the immune modulating effects of prebiotic carbohydrates. Dr Garssen discussed the elements of immune regulation particularly in the intestine through the balance between T<sub>H1</sub> and T<sub>H2</sub> as modulated by T<sub>REG</sub>. An imbalance in this regulation may give rise to either hyper-immune responsiveness resulting in allergy, autoimmunity, and chronic inflammatory diseases, or hypo-immune responsiveness resulting in infections as well as tumors and metastasis. The balance between T<sub>H1</sub> and T<sub>H2</sub> is a function of age as influenced by many factors including diet. In the case of breast fed infants, human milk contains numerous compounds with immunological properties. Of particular interest are oligosaccharides and prebiotics in human milk. While these oligosaccharides in human milk are not commercially available, a synergistic mixture of specific prebiotic polysaccharides has been developed with functional properties similar to those in human milk, namely, 90% short chain galacto-oligosaccharides (scGOS) and 10% long chain fructo-oligosaccharides (lcFOS). Dr Garssen described their in vitro and in vivo experiments which demonstrated that scGOS/lcFOS not only acts as prebiotics, i.e., induction of probiotic bacteria (bifidobacteria), but is capable actually modulating the immune system. Clinical trials in infants showed that scGOS/lcFOS could protect formula-fed infants against infections during the first 6 months of life and decrease IgE in high-risk infants. Dr Garssen concluded that nutritional ingredients such as non-digestible carbohydrates are essential for a healthy immune system in infants. The last speaker in the session was Dr Sunil Sazawal of Johns Hopkins Bloomberg School of Public Health. He again briefly described their study that he reported earlier among 1-4 yr old children in a peri-urban community in outskirts of New Delhi, to evaluate the effect of milk fortified with multiple micronutrients (especially iron and zinc) on morbidity, anemia, iron status, growth and physical activity in comparison with the same milk without fortification. The results showed improvement in mean body iron stores, hemoglobin levels and erythrocyte iron markers among children consuming fortified milk. Likewise, there was significant improvement in the height, weight, WAZ, and HAZ scores as well as improved physical activity as shown by leg and arm activity scores. Dr Sazawal concluded that milk is a well accepted and term health benefits, especially for infants and also for the elderly. 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The final session of the conference dealt with some examples of Asian foods and their effect on immunity and immune response. The first speaker was Prof Rina Yu, of the Department of Food Science and Nutrition, University of Usan, Korea, who discussed their studies on soybean saponin and capsaicin against inflammation-associated pathologies. After summarizing past in vitro and in vivo studies on anti-tumor activities of soybean saponins, Prof. Yu described their recent studies to demonstrate anti-inflammatory activities of soybean saponins. They demonstrated that soybean saponins could elicit anti-inflammatory activities in inflammatory-stimulated macrophages and that their action is mediated by NF-κB inactivation. Their in vivo studies in mice showed that soybean saponins could suppress inflammation-associated lung tumor metastasis. Likewise, their in vivo and in vitro experiments demonstrated that capsaicin suppresses obesity-induced inflammatory responses by inhibiting MCP-1 gene expression and protein release from obese-mouse adipose tissues and by suppressing the inflammatory responses of adipose tissue macrophages. At the same time they showed that capsaicin improves obesity-induced insulin resistance. Prof Yu concluded that soybean saponin and/or capsaicin may be useful phytochemicals against inflammation-associated pathologies. The next speaker was Prof Chan Son Ha of the National University of Singapore, who discussed their studies on Lycium Barbarium fruit (Chinese Wolberry) which contains fat-soluble and water soluble components alleged to stimulate T-cell response. Their studies showed that polysaccharide protein complex from Lycium Barbarum (LBP) could enhance immunity by activating T-lymphocytes. They found that crude LBP as well as fractions LBPF4 and LBPF5 isolated from Lycium Barbarum significantly stimulated mouse splenocyte proliferation, targeting T cells but not B cells. Likewise, these fractions could induce expression of IL-2 mRNA, IFN-γ mRNA, and TNF-α mRNA. Further studies showed that LBP and LBPF 1-5 stimulated macrophages and dendritic cells to produce cytokines, while administration of LBP in vivo induced T lymphocyte proliferation. Prof. Chan concluded that activation of T-lymphocytes by LBP may contribute to its immune-enhancement function. The next speaker was Dr Dayong Wu of the Nutritional Immunology Laboratory, Jean Mayer USDA Human Nutrition Research on Ageing at Tufts University, USA, who discussed their studies on white bottom mushroom (WBM) and its effect on immune functions in mice. Mushrooms have been shown to possess anti-tumor and anti-microbial properties probably due to their ability to modulate immune cell functions. Their studies on the effect of dietary supplementation with WBM showed dose-dependent enhancement of natural killer (NK) cell activity and production of interferon (IFN) and tumor necrosis factor (TNF) but not IL-2 and IL-10 in splenocytes, indicating that WBM supplementation enhances NK activity through increase in INF and TNF production. In another study, Dr Wu showed that WBM supplementation in mice dose-dependently enhanced the expression of maturation markers of bone marrow-derived dendritic cells and their antigen presenting function. They concluded that WBM may have a potential in enhancing both innate and T-cell mediated immunity, leading to a more efficient defense mechanism against microbial invasion and tumor development. The next topic of discussion was given by Dr Ammu K Radhakrishnan of the International Medical University, Malaysia, who discussed their studies on the effect of vitamin E supplementation on immune response to tetanus.
toxoid immunization. In their study on the immunomodulatory effects of orally administered tocotrienols and tocopherols on the mouse immune system upon immunologic challenge with tetanus toxoid (TT), they found a significant increase in lymphocyte proliferation in vitamin E-treated mice compared to controls. Anti-TT antibody production was also significantly augmented. Cultured splenocytes from vitamin E-supplemented animals produced significantly higher levels of IFN-γ. In their subsequent study among healthy volunteers immunized with TT vaccine, supplementation with tocotrienol rich fraction (TRF) increased plasma vitamin E level compared to placebo. Anti-TT IgG production was also significantly increased. Dr Radhakrishnan concluded that supplementation with TRF or vitamin E from palm oil has immunostimulatory effects and potential clinical benefit for boosting immune function. The last speaker of the conference was Dr Ingrid Surono of SEAMEO TROP-MED RCCN, University of Indonesia, Indonesia. Dr. Surono described their studies on the probiotic properties of lactic acid bacteria isolated from dadih, an Indonesian traditional fermented buffalo milk. The effect of each of three probiotic strains of lactic acid bacteria (L. casei, shirota group, L. plantarum IS-10506, and L. plantarum IS-20506) fed to rats on viability (fecal lactic acid bacteria), as well as humoral immune system (IgA and sigA) in vivo was determined. They found that rats fed with the probiotics significantly increased fecal lactic acid bacteria and secretory IgA compared to controls, and the highest increase was found in rats fed with L. plantarum IS-10506. Dr Surono concluded that L. plantarum IS-10506 isolated from dadih is a potential probiotic.

The 2-day conference ended with the summary given by Dr Rodolfo Florentino, Nutrition Foundation of the Philippines, who concluded that while the symposium brought out that much has already been learned about the very complicated immune system, it was apparent that there is a need for much more research on the influence of diet and nutrition in immunity. Randomized controlled trials with larger number of subjects and improved methodologies were emphasized in the conference. Asia with its large variety of foods known to have functional properties offers a fertile ground for research for functional ingredients with potential for improving innate and adaptive immunity. On the other hand, it was realized that, with the vast knowledge already learned about diet and immunity, there is now an enormous opportunity to translate the results of scientific studies into clinical and public health practice.

AUTHOR DISCLOSURES
Rodolfo F Florentino, no conflicts of interest.
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飲食、營養和免疫的研討會

2008 年 4 月在新加坡舉行一個飲食、營養和免疫的研討會，回顧當前的科學訊息，特別是在嬰兒期的免疫系統發展，以及飲食、運動、老化在免疫能力上的角色，還包括相關的分子生物層次。尤其是鋅、鐵、維生素 D、維生素 E 和長鍵多元不飽和脂肪酸，皆是能提高免疫特性的重要營養素，另外，維持腸道健康微生物群落的重要，也引領了益生菌及益生源的概念來做討論。而數篇研究，則提出一些普遍的亞洲食物，如大豆、枸杞、蘑菇等，可能應用於預防和治療有關發炎反應的疾病。

關鍵字：飲食、免疫系統、微量和巨量營養素、益生菌及益生源、亞洲食物