Immunonutrition - Enhancing Tumoricidal Cell Activity

Executive Summary

By: Susan S. Percival, Stefanie A. Nelson and John A. Milner

Purpose of the Workshop

Susan Percival, Nutritional Sciences Research Group (NSRG), Division of Cancer Prevention (DCP), National Cancer Institute (NCI)

This workshop was designed around mounting information linking immuno-suppression with malignancy, the ability of bioactive food components to enhance certain aspects of immunity, and evidence that dietary habits can influence cancer risk and tumor behavior. This workshop brought together immunologists, cell biologists, and nutritionists to discuss issues surrounding diet, the immune system, and cancer prevention. Two types of tumoricidal cells, natural killer (NK) and γδ T, were given special attention as potential targets for bioactive food components. Dr. Percival asked participants to discuss current knowledge with a goal of identifying the strength of evidence and research gaps.

Welcome

John Milner, NSRG, DCP, NCI

Historically, nutrition has been proclaimed to have a critical role in achieving immunocompetence. Today scientists are beginning to categorize those dietary compounds, bioactive food components, that may impact immunity and to identify their possible sites of action. To fully understand the roles of bioactive food components in cancer prevention, additional information will be needed to define effective concentrations of these food components and their interaction with environmental and genetic factors. This information will be critical for the identification of responders and non-responders to dietary intervention.

NCI's research strategy is based on the 3 D's – Discovery, Development, and Delivery. Today, much of nutrition and cancer prevention research is focused on the discovery mode, i.e., identifying actions of bioactive food components, under what circumstances these actions occur, and how they affect physiological relevant processes. The next step will be to move these discoveries into the development mode; that is to explore the utility of using benchtop research findings in a clinical situation. The final step comes from the testing and delivery of information in larger populations. This phase thus ideally builds on a wealth of preclinical and clinical evidence. Several studies including the Selenium and Vitamin E Cancer Prevention Trial (SELECT) and Women's Initiative Nutrition Study (WINS), are underway to examine if specific populations respond to dietary intervention. Ultimately information arising from these trials will be made public, perhaps in the form of dietary guidance such as the food pyramid of some other public health message. Clearly the goal of the overall process is to translate basic information to improve the health of all people regardless of their current status.

A major goal of this workshop is to assist in identifying gaps in knowledge and determine how NCI can assist in stimulating research in this area, perhaps through Requests for Applications (RFAs) or Program Announcements (PAs), although other approaches may

be used. Another goal of the workshop is to prepare an executive summary of this meeting, which will be posted at the Nutritional Science Web site (http://programs-resources/groups/ns) and perhaps published elsewhere. This will allow those unable to participate in the meeting to learn what was discussed, which will help to stimulate research. Finally, it should be noted that funding opportunities are available for high quality investigator-initiated applications that will help advance the science, regardless whether or not in response to a RFA or PA.

Introductory Lecture

Moderator, Susan S. Percival

How Compelling Are the Data Regarding Nutritional Influences on Our Immunity?

M. Eric Gershwin, University of California, Davis

Although adequate nutrition has an essential role in immune function, data showing it directly improves immunity and alters carcinogenesis does not exist. Nevertheless, nutrients can modulate innate immunity, influencing barrier functions, surveillance of mucosal surfaces, and the function of NK and polymorphonuclear cells. At supra-physiological exposures, however, some bioactive food components may become hazardous. It is also possible that toxic contaminants might surface for isolates or extracts when consumed in sufficiently high amounts. Finally, some food components may potentially become mutagenic when consumed in high amounts. Most studies examining the effects of nutrition on the immune system has focused on the adaptive immune response. There is a dearth of such research for the innate immune response, for which many of the components, along with their functions, have been only recently described.

Fighting infection is recognized to involve many different types of white blood cells. Initially, macrophages ingest invading infectious agents, followed by antigen display on macrophage surfaces. The antigens are presented to other immune cells, including B cells that are stimulated to produce antibodies against the invading organism. The antigens on the surface of the macrophages also induce helper T cells to proliferate, followed by activation of killer T cells, which multiply and destroy invading infectious agents.

Many substances, including flavonoids, omega-3 fatty acids, zinc, and vitamin C, are alleged to strengthen the immune system. In their role as antioxidant, these nutrients may improve the inflammatory response and thus may help suppress tumorigenesis. To enhance immune function, bioactive food components must: (1) increase antibody production from B-cells to eliminate extracellular pathogens; (2) enhance the ability of macrophages to phagocytose; (3) enhance the ability of NK cells to identify and destroy dysfunctional cells, such as infected or cancerous cells; and (4) increase T cell production of cytokines such as interferon-γ (IFNγ) and tumor necrosis factor (TNF).

Although it is unlikely that nutritional supplements can improve already strong immune systems, nutrients may be useful for improving immune function in populations with poor nutritional status. In populations with specific nutrient deficiencies, increased rates of usually rare infections are often observed. Immune system function also declines with age; nearly 50 percent of people older than 60 years do not respond to the pneumococcal vaccine, perhaps attributed to poor nutrition or nutrient deficiencies in this population. Historically, studies of the military have shown that stress combined with

poor nutrition is responsible for more casualties than the battlefield. Attempts to improve immune system function using nutritional supplements might be particularly beneficial to these types of populations.

There are many examples of bioactive foods that influence the immune system; for example, beneficial health effects have been attributed to mushrooms for millennia. Mushrooms extracts are strongly bioactive and, *in vitro*, can inhibit growth of some tumors. This effect is attributed to polysaccharides and may occur through their ability to enhance the numbers and/or functions of macrophages, NK cells, and subsets of T cells. Some of this bioactivity may, however, be harmful, because certain kinds of mushrooms are known to induce potentially harmful mutations. This may be a particular issue in children and requires further study. Spirulina, a blue-green alga, is a nutritional supplement with claims that it strengthens the immune system. Specifically, there are indications that it can enhance phagocytic functions of macrophages, increase antibody-dependent cell-mediated cytoxicity of NK cells, strongly induce IFNγ, and moderately stimulate interleukin-4 (IL-4) and IL-1β. It is particular rich in anti-oxidants and potentially beneficial sulfalipids.

Other immune system components to target with potential immune function enhancers include IL-6, which promotes tumor cell growth and has been implicated in tumor progression and chemoresistance. Anti-IL-6 monoclonal antibodies induce apoptosis and regression of human prostate cancer cells in nude mice; inhibition of IL-6 may be an important cancer-fighting strategy. IL-6 production can be decreased by blocking the transcription factor, NFκB that regulates IL-6 gene expression. Blocking NFκB suppresses angiogenesis, invasion, and metastasis. Many plant extracts, including flavonoids from soy and cocoa, have potential NFκB inhibitory activity. Other potential targets include transforming growth factor-β (TGF-β), levels of which can also be modulated by cocoa flavonoids, and activation of γδ T cells by certain bioactive components found in tea.

Nutrients can affect pathogen genotype and virulence and possibly vertical transmission. The nutrition-immunity-cancer model is, therefore, more complex than originally thought. For an otherwise healthy immune system, it is unlikely that nutritional therapy can produce enhancement. Nevertheless, during periods of stress and for the very young and the elderly, there is a basis for a rigorous research program designed to improve and enhance immune function.

Discussion

Dr. Simin Meydani commented that, in addition to how the immune system affects cancer, it is also necessary to consider the effects of tumors on the immune system; for example, in some cases, tumors secrete immunosuppressive agents. The effects of nutrients on this activity are unknown. Dr. Gershwin answered that a literature search did not identify any evidence that nutrients affect or alter tumor immunosuppressive activities.

Dr. Susanna Cunningham-Rundles asked participants to consider the effects of nutrition on redistribution of cells for control of cancer. Nutrition may not be able to affect *de novo* production of cells, but there are diseases (neutropenia, platelet disorders) in which failure to release cells from bone marrow is a significant problem. Correcting distribution of the appropriate cells could be an effective treatment. Dr. Gershwin agreed that this would be a good strategy if redistribution leads to a biological effect. Several clinical situations have shown that supplements can affect production, but whether this affects malignancies is unclear.

Dr. Percival asked about the Spirulina studies, specifically, whether levels of IL-4, IFNγ, and IL-2 were assayed in plasma or from secretion of cultured white blood cells. Dr. Gershwin answered that both sources were assayed.

Session 1. Role of Immunocompetence in Cancer

Moderator: Kevin Fritsche

Biology of γδ T Cells: What Is the Relationship Between γδ T Cells and Cancer? Will an Increased Number and/or Function of γδ T Cells Result in Lower Cancer Incidence?

Wendy Havran, The Scripps Research Institute

White cells that may assist in explaining a host of inflammatory diseases is the γδ T cell. γδ T cells are similar to αβ T cells, but have different ontogeny and are linked to different signaling pathways. Distribution of γδ T cells is also unusual; these cells are relatively rare in normal blood and lymphoid organs, but are the predominant T cell population in epithelial tissues. γδ T cells also differ from αβ T cells with respect to antigen recognition. Rather than recognizing specific antigens, γδ T cells appear to recognize a common "self" antigen up-regulated by trauma or disease. Despite the potential for a highly diverse array of receptors (greater than that observed for αβ T cells), often only a single monoclonal receptor is used, especially in epithelial tissues. Although no definitive description of γδ T cell antigens has been developed, characteristics of these antigens include low molecular weight non-peptide molecules, products of infection or cell stimulation, microbial metabolites, alkylamines, and aminobisphosphonates. A paradigm for γδ T cell antigens is thus a critical area of investigation.

Several disease states are associated with increased numbers of γδ T cells, although the function of the cells in these conditions is not clear. Elevated γδ T cell numbers are observed in bacterial and viral infections, adenocarcinoma, autoimmune diseases, and skin conditions such as psoriasis and wound healing. Recent experiments, using a mouse model for wound healing, demonstrated that at the wound site, γδ T cell round up, lose contact with epidermal cells, proliferate, and are activated. Activated γδ T cells appear to facilitate wound re-epithelialization and may stimulate production of keratinocyte growth factor (KGF), a potent mitogen for keratinocytes and other epithelial cells. T cell receptor (TCR) δ -/- mice have a decreased rate of wound healing; addition of activated γδ T cells to culture skin from TCR δ -/- mice restores proliferation of the TCR δ -/- keratinocytes. Although these activities are beneficial for wound healing, some tumors also possess KGF receptors; thus, activated γδ T cells could potentially stimulate tumor growth and metastasis.

Several mouse models have demonstrated anti-tumor activities for γδ T cells. *In vitro*, γδ T cells lyse a variety of tumor cell lines and ex vivo tumors, express perforin, granzymes, and granulysin, and induce apoptosis via the FasL-Fas pathway. *In vivo*, TCR δ -/- mice show rapid tumor onset and increased tumor incidence. Anti-tumor activity appears to be mediated by TCR and NKGD interaction with ligands and through IFN-γ production to enhance cytotoxicity. Human γδ T cells have been observed to lyse many tumor cells *in vitro*, and patients with tumors of epithelial origin have increased numbers of γδ T cells in peripheral blood and among tumor-infiltrating lymphocytes. Potential strategies for γδ T cell based immunotherapy include *in vivo* use of stimulating ligands to expand and activate γδ T cells and isolation of γδ T cells followed by *in vitro* expansion and adoptive

transfer of γδ T cells into the patient. Potential stimulating ligands include synthetic phosphoantigens and aminobisphosphonates.

In vitro and animal studies are aimed at identifying the mechanisms of the γδ anti-tumor responses and to optimize strategies for γδ T cell mediated immunotherapy. These results indicate that γδ T cells may be useful for immunotherapy to induce anti-tumor responses.

Discussion

Dr. Pamela Fraker asked where γδ T cells appear in the scheme of lymphopoiesis. Dr. Havran responded that they appear early in ontogeny and have neither CD4 nor CD8 antigens and thus are derived from double negative thymocytes. γδ T cells do not undergo the traditional T cell selection process to control for the receptors they express, but instead appear to have a hard-wired specificity. γδ T cells do not recognize foreign antigens; instead, they recognize consequences of stress, including traumas such as cuts, ultra violet (UV) radiation, tumors, infection, or other insults to the skin. These traumas up-regulate self antigens, a common signal of distress. The heat shock proteins could be candidates for the recognized antigen.

Dr. Fraker commented that zinc oxide was used to promote wound healing, and zinc pyrethroids for psoriasis, but these treatments have been dropped, perhaps due to questions concerning how they impact the immune system. Skin deteriorates with age — could zinc inappropriately up-regulate γδ T cells or is this just an effect of epithelial cell health? Dr. Havran answered that she was not aware of any studies on this, since γδ T cells are resident in epithelium and most immunologists do not study this tissue.

Dr. Kevin Howcroft asked whether γδ T cells contribute to tumor formation or stimulate proliferation. Dr. Havran answered that a subset of tumors expresses KGF receptor, so in theory γδ T cells would promote growth of these tumors. This has not been shown, in fact, γδ T cells are cytotoxic to tumor cells *in vitro* and *ex vivo*.

Dr. Milner asked whether compensatory mechanisms occur in knock-out mice to offset for their loss of γδ T cells and whether this affected their phenotype. Dr. Havran answered that in these mice, αβ T cells move into the skin, where they are not normally found. They do not compensate for the function of γδ T cells because they do not make KGF and do not respond to antigens on the wounded keratinocyte.

Biology of NK Cells: What Is the Relationship Between NK Cells and Cancer? Will an Increased Number and/or Function of NK Cells Result in Lower Cancer Resistance?

Stephen Anderson, NCI, Frederick

NK cells have multiple roles in the innate immune response, including tumor surveillance and response to viral infections. NK cells are circulating lymphocytes with distinct markers and morphology and are cytotoxic without prior sensitization. Infected or pre-cancerous cells are targeted by NK cells, which promote cell lysis by secreting lytic granules, which release perforin and serine proteases into the target cells. NK cells can also induce tumor cell death by promoting apoptosis through TNF-related pathways.

NK cells have inhibitory receptors that recognize self class I major histocompatibility complex (MHC) antigens and detect a lack of self antigens on target cells, rather than recognizing foreign antigens. NK cell receptors are encoded by the KIR genes in humans and by the Ly49 gene family in mice. Both mouse and human receptors have

intracellular domains that interact with Shp-1 phosphatase, which prevents NK cells from killing healthy self cells. NK cells also have activating receptors to promote NK cell-mediated lysis. Viral infection or carcinogenic changes in a cell result in loss of self antigens, but may lead to up-regulation of stress molecules recognized by NK cell activating receptors.

Genetic variation in NK cell receptors results in differences in sensitivity to pathogen infection and in the tendency to develop autoimmune conditions. In mice, for example, B6 mice are more resistant to cytomegalovirus (CMV) infection than BALB/C mice because activating receptors on B6 NK cells recognize the m157 molecule expressed on CMV-infected cells. BALB/C mice have a smaller and simpler Ly49 gene cluster, limiting their ability to respond to CMV infection. Although increased ability of NK cell activating receptors to respond to infection is beneficial, human KIR haplotypes encoding more activating NK receptors may indicate a greater susceptibility to development of autoimmune diseases.

Not all types of receptors are found on all NK cells; instead, receptors show variegated expression by a probabilistic mechanism. Variegated expression allows NK cells to detect selective loss of antigen expression on the surface of target cells, thus preventing infected cells from evading destruction and preventing NK cells from inappropriately destroying healthy cells. Variegated receptor expression is also characteristic of sensory systems, such as the olfactory and taste systems. In mice, a switch model of Ly49 activation has been identified, including a promoter that allows transcription in either the forward and reverse directions. When transcription occurs in the reverse direction, the coding region of the receptor is not transcribed. Each switch has a different probability of being in the "off" or "on" position.

Three examples of nutritional modification of NK cell activity also were described. Lactobacilli enhanced NK cell activity in one study but had no effect in another. Zinc is required for proper clustering of KIR receptors, but one study showed enhancement of NK cell activity by zinc, whereas the other showed a decrease in activity. In two studies, arginine increased NK cell activation but had no effect on tumor growth. These studies, however, examined the effect of arginine on solid tumors, and NK cells are more active against circulating tumors and tumor metastases.

NK cells are also important mediators of anti-tumor immunity. This is clearly displayed in transgenic mice with decreased amounts of NK cells. Such mice have decreased resistance to lung metastases and tumor outgrowth. Furthermore, protection from the development of carcinogen-induced tumors is dependent on NK cells.

Discussion

Dr. Meydani commented that up-regulation of the immune system in general does not always result in autoimmune disease; it depends instead on the types of B cells involved. Dr. Esmail Tabibi noted two studies using lactobacilli against cancer; one study used lactobacilli as cancer treatment and was somewhat effective, but the other, which used lactobacilli for prevention, was not.

Dr. Suzanne Klimberg stated that ketamine, a common animal anesthesia, can inhibit NK cell activation for up to two weeks. If ketamine is used during tumor implantation, results concerning NK activity against the tumor may be misleading. Dr. Anderson commented that some dietary components that inhibit NK cell activity include steroids, fats, and alcohol.

Dr. John Erdman observed that high dietary zinc can compete for copper absorption, so results observed for experiments with zinc may reflect this. Dr. Milner commented that arginine might suppress tumor growth, but ornithine, a breakdown product of arginine, appears to stimulate growth. A marked increase in growth of mammary tumors is observed in response to ornithine. Variability in arginase activity could impact susceptibility to these tumors. This example relates to the idea of balance—if one branch of the immune system is activated (or suppressed), how does this affect other branches? Dr. Anderson responded that this is why targeting specific receptors or activities may be a safer strategy. Dr. Milner addressed the need for better studies to determine optimal nutrient concentrations. Dr. Kevin Fritsche remarked that within a given population, increasing T cell activity or TNF concentrations will be beneficial to some people, but not to others. Genetic variability will influence "optimal" nutrient concentration.

Dr. Fraker commented that high doses of zinc taken intranasally (Zicam) to treat a cold has led to loss of sense of smell in some users. This is likely caused by the ability of nanomole quantities of zinc to induce apoptosis. Using 50 to 100 times the Recommended Dietary Allowance (RDA) of a nutrient is usually not advisable. Dr. Meydani added that dose response studies must consider the type of nutrient and the part of the immune system that nutrient affects. In the elderly, who are more prone to autoimmune disease, vitamin E increased T cell function, but did not have an effect on auto-antibody formation. Dr. Bobbi Langkamp-Henken added that although pharmacological doses of a nutrient may be more likely to be harmful, many Americans suffer from specific nutrient deficiencies, and returning these people to normal physiological nutrient status may benefit their health.

Session 2. Models of Dietary Factors, Immunity and Cancer

Moderator: Simin Meydani

Is the Increase in γδT Cell Priming by Dietary Alkylamines Sufficient to Prevent Cancer? What Other Components of the Diet Prime γδT Cells?

Jack Bukowski, Brigham and Women's Hospital and Harvard Medical School

γδ T cells may serve as a manipulable first line of defense against infection, expanding up to 50-fold in response to bacterial, viral, and parasitic infections. Transfer of human PBMC containing human γδ T cells into Severe Combined Immune Deficient (SCID) mice, followed by challenge with bacteria, results in γδ T cell-mediated antibacterial activity within 24 hours; this was unexpected because, in culture and in this SCID model, γδ T cells require 6 to 7 days to proliferate to significant levels. This antibacterial activity correlates with 3-fold increased serum IFN-γ in γδ T cell sufficient SCID mice, as compared to SCID mice given human PBMC without γδ T cells. Thus, γδ T cell-mediated antibacterial effects are most likely dependent on very early IFN-γ secreted by γδ T cells. In humans, γδ T cells comprise 2 to 3 percent of the peripheral blood, mucosal and skin populations, providing a large population of functional T cells. γδ T cells have been shown to produce large concentrations of cytokines within 24 hours of infection, thus promoting an early response to infection.

γδ T cell antigens include alkylamines and alkyl phosphates. Alkylamine antigens are secreted by both pathogenic and commensal bacteria and are found at millimolar concentrations in human urine, breast milk, amniotic fluid, and vaginal secretions. Dietary factors are likely to contribute to alkylamine concentrations, and potential sources of alkylamine antigens include tea, apple skins, red and white wine, some

mushrooms, and cucumbers. Alkylamines in high concentrations stimulate expansion of and cytokine secretion by γδ T cells. Such activity requires cell-cell contact, but the antigen presenting molecule is not any of the classical or non-classical MHC or CD1 molecules. TCR transfection experiments show that the Vγ2Vδ2 TCR is required for stimulation, indicating that this is a specific TCR-dependent phenomenon. Alkylamine antigens will prime γδ T cells to destroy tumor cells. γδ T cells can be primed with alkylamine antigens to react to different antigens. Primed γδ T cells provide nearly all of the very early IFN-γ response to heat-killed bacteria and lipopolysaccharide (LPS). This proliferation and cytokine secretion by primed γδ T cells in response to LPS stimulation is almost totally dependent on IL-12. Thus, LPS-induced IL-12 secretion by monocytes causes γδ T cells to expand and secrete cytokines, but only if these are first primed with alkylamines or alkyl phosphates. Thus the physiologic role of these ubiquitous alkylamines is most likely to keep γδ T cells primed so that they may respond more efficiently to cytokines such as IL-12 that is induced by bacterial stimulation of monocytes. This enhanced reactivity to IL-12 may explain why alkylamine-primed γδ T cells protect against a wide array of bacteria that do not secrete alkylamines or any other γδ T cell antigen.

Tea contains L-theanine, an amino acid metabolized to glutamic acid and ethylamine after consumption. Because tea contains l-theanine, a dietary precursor for the alkylamine antigen, experiments were performed to determine if tea could enhance natural immunity in humans by priming γδ T cells. Ten unmatched volunteers were given five to six cups of tea or coffee (control) per day. Tea drinking did not cause *in vivo* expansion or secretion of cytokines by γδ T cells, but did enhance response to bacterial challenge *ex vivo*. No response was observed in coffee drinkers, eliminating caffeine as a mediator of this effect. The enhanced response observed with the primed γδ T cells is IL-12 dependent; IFN-γ activates monocytes, which secrete IL-12, which in turn promotes γδ T cell expansion. Activated monocytes also enhance the adaptive immune response. Because tea drinking appears to prime γδ T cells, it is possible that tea also could decrease cancer incidence. Prospective double blind, placebo-controlled studies will be difficult, because at least six cups of tea per day were needed to see an effect. Production of a capsule, which contains defined amounts of various tea extracts, may help solve this problem.

It has been shown that exposure of PBMC or cloned gamma delta T cells to alkylamines results specifically in gamma delta T cell expansion, Th1 cytokine secretion, cytotoxic activity, and an increase in the efficacy of antimicrobial protection. Research needs to be done to show that tumoricidal activity is also enhanced.

Discussion

In response to a question from Dr. Fraker, Dr. Bukowski clarified that his human tea studies showed an increase in the number of γδ T cells producing IFN-γ in response to *ex vivo* stimulation with bacteria, not in the number of circulating γδ T cells.

Dr. Elizabeth Jeffery asked whether green or black tea was used and whether levels of l-theanine varied among different teas. She also asked whether epigallocatechin gallate (EGCG) could interact with l-theanine to affect γδ T cells. Dr. Bukowski responded that there was no evidence for interaction of EGCG with l-theanine, but instead EGCG may augment tumor surveillance in general. Green tea generally has more l-theanine than black tea, but at this point there is no defined dose for l-theanine. Dr. Jeffrey asked how Dr. Bukowski could distinguish between the effects of EGCG and l-theanine in cancer. Dr. Bukowski answered that he cannot distinguish between these effects at this time.

A participant asked what sort of pattern recognition receptors are on γδ T cells and whether they are a good source of IL-12 early in infection. Dr. Bukowski answered that γδ T cells are important in early infection. Toll-like receptors (TLR) 2 and 4 are required for pattern recognition, but are not found on γδ T cells. He added that his studies show that primed γδ T cells are more sensitive to IL-12 than are primed αβ T cells.

How Can We Best Show Directly that Immunity Influenced by Diet Modifies Cancer: Studies with Dietary Fat in a UV-Carcinogenesis Model?

Homer Black, Baylor College of Medicine

UV radiation is a potent immunosuppressant that promotes acceptance of highly antigenic transplanted tumors in mice. Dietary fats, in particular omega-3 and omega-6 fatty acids, influence UV-induced carcinogenesis. Increased intake of omega-6 fatty acids reduce tumor latent periods and increase tumor multiplicity. Increased levels of omega-3 fatty acids increase tumor latent periods and reduce tumor multiplicity. UV-induced carcinogenesis in mice pre-fed a low fat (corn oil) diet, which was then switched to a high fat diet, showed increased tumor multiplicity; the opposite was true for mice pre-fed a high fat diet and switched to a low fat diet. Other studies have suggested that the promotion stage of carcinogenesis might be immunologically modulated and that an essential fatty acid deficiency was associated with protection from UV-initiated tumor outgrowth.

Suppressor T cell function is prostaglandin E_2 (PGE_2) dependent and prostaglandin synthesis inhibitors abrogate UV suppression of contact hypersensitivity. Dietary lipid levels are related to plasma concentrations of PGE_2, with omega-6 fatty acids associated with higher amounts of PGE_2 as compared to omega-3 fatty acids. Omega-6 fatty acids also increase inflammatory response and reduce delayed-type hypersensitivity (DTH) response, while omega-3 fatty acids are correlated with lower inflammatory response and higher DTH response. To determine if dietary lipids influence UV-carcinogenic expression through immune function, DTH response of mice fed low and high lipid diets (0.75 percent vs. 12 percent corn oil) was examined. Before UV irradiation, mice fed low fat diets had a 5-fold higher DTH response than those fed a high fat diet. After initiation of UV treatment, DTH response in mice fed a low fat diet continued through week 9, while no response was seen in mice receiving a high fat diet. UV-induced reduction of T cell population, in general, showed no effect of diet, but a high fat diet resulted in a greater increase in the population of I-J+ cells, a presumptive suppressor cell subset.

The influence of dietary lipid on immune status was also examined in tumor transplantation studies. Transplanted tumors, derived from UV-induced squamous cell carcinomas, were rejected at higher rates in animals fed low fat diets as compared to those fed the high fat diet. By 20 days after tumor transplantation, fewer tumors were sustained in animals fed a low fat diet as compared to those consuming the high fat diet. Median tumor rejection time was also significantly shorter for animals fed the low fat diet. Rejection occurred more quickly if recipient animals were not exposed to UV radiation, underscoring the ability of UV radiation to suppress immune response. Median tumor rejection time in UV-irradiated, low fat-fed mice was approximately 21 days, similar to rejection rates in non-irradiated animals. Median tumor rejection time for UV-irradiated animals fed the high fat diet was 63 days.

To determine whether dietary fat affects primary UV-induced tumor formation through modulation of the immune response, mice were fed either a low or high fat diet. Lymphocytes from animals receiving 11 weeks of UV-irradiation and fed the high fat diet were transferred to UV-irradiated mice fed the low fat diet at 9 and 12 weeks of their 11

week UV regimen. Mice fed a low fat diet had a tumor latent period of 21.6 weeks. Tumor latent period for mice fed the high fat diet was 15.8 weeks. Animals fed a low fat diet who received lymphocytes from UV-irradiated, high fat fed animals had a tumor latent period of 18.5 weeks; clearly demonstrating that dietary lipid could have significant effects upon specific immunologic responses affecting modulation of carcinogenic expression. To determine if this immunological effect of dietary fat on UV-induced tumor formation could, itself, be modulated, cell free extracts were prepared from T-14 cells (a cell line derived from UV-induced squamous cell carcinomas of the animal model) and injected into mice for the first 3 weeks of the 11-week UV protocol. Animals were then challenged with T-14 cells at 15 weeks. Immunized animals had a significantly greater tumor rejection rate than non-immunized mice. Immunization also protected mice from the immune-suppressing effects of a high fat diet; these mice had tumor volumes similar to animals fed a low fat diet. The T-14 immunogen also was injected into animals during the first 3 weeks of an 11-week UV protocol to assess primary tumor formation. Animals fed high fat diets had tumor latent periods of approximately 19 weeks, while immunized animals had a tumor latent period of 21 weeks. This study demonstrates that a major mode of action of dietary fat on UV carcinogenic expression occurs via modulation of immune pathways and occurs at a time when the host animal has already been immune compromised by UV radiation.

Regardless of specific mechanism(s), it is clear that a major mode of action of dietary fat on UV-carcinogenic expression occurs *via* modulation of immune pathways; this effects is manifested at a time when the host animal has already been immuno-compromised as a result of UV radiation; and the magnitude of this post-UV fat effect may, itself, be modified through immunologic manipulation.

Discussion (Drs. Bukowski and Black)

Dr. Fritsche commented that in Dr. Black's model, PGE_2 is the primary driver of modulation of the immune response and tumor latency. Changing from a high fat to a low fat diet affects PGE_2 concentrations, so Dr. Fritsche asked whether other PGE_2 modifiers, such as aspirin, could give the same results. Dr. Black answered that they have not yet looked at aspirin, but would expect that this would be case. They have assessed the use of Celebrex as a chemopreventive for UV-induced carcinogenesis. Dr. Fritsche added that data suggest this mechanism works through changing tumor latency, not necessarily through affecting tumorigenesis, and asked if this is a T suppressor phenomenon, impacting an antigenic tumor, why there is no difference in tumor incidence. Dr. Black commented that tumor incidence curves were shown with respect to tumor latency and agreed that it would have been useful to look also at tumor multiplicity.

Dr. Anderson asked whether tumor latency could be influenced by the higher energy of a high fat diet affecting tumor growth rates. Dr. Black answered that the high and low fat diets had equal numbers of calories, so the differences were probably not purely a caloric effect. Eicosanoid metabolite production is related to dietary fat intake and serves as a gatekeeper for cytokine production.

Dr. Anderson asked Dr. Bukowski whether he had looked at the effects of tea on γδ T cells in hyper-immune gut syndromes such as Crohn's disease or irritable bowel disease. Dr. Bukowski thought that tea might have an effect on these conditions. In mice, enhancing γδ T cells ameliorates autoimmune diseases, although he was not sure of the effect on conditions like Crohn's or irritable bowel disease. Dr. Meydani asked

whether effects of tea were observed for T cells besides γδ T cells. Dr. Bukowski answered that this has not been analyzed yet.

Dr. Tabibi commented that NIH's Rapid Access to Interventional Development (RAID) pilot program (http://grants2.nih.gov/grants/guide/notice-files/NOT-CA-04-019.html) could help in the production of Dr. Bukowski's tea capsules. Dr. Milner added that RAID and NCI's Rapid Access to Preventive Intervention Development (RAPID) (http://programs-resources/programs/rapid) are programs designed to help investigators determine whether a compound has merit in cancer prevention or treatment. These programs help with preclinical studies, bulk formulations, good manufacturing processes, and toxicology among others. Dr. Milner added that he was skeptical about the efficacy of six cups of tea per day, because epidemiological data was not overly compelling. Dr. Bukowski agreed that this was a problem, adding that the tea capsules would help solve this problem. He added that in his pilot study, subjects received 200 mgs of l-theanine per day, which was the amount obtained from the tea given to the subjects.

Session 3. Dietary Modifiers of Tumoricidal Immunity

Moderator: Pam Fraker

How Are Dietary Signals (Probiotics and Prebiotics) Processed by Gastrointestinal Cells To Effect Measurable Changes in Immune Parameters Systemically?

Norman Hord, Michigan State University

Nutritional interventions to improve immunosurveillance may be an effective strategy for cancer prevention. One approach may be to change the intestinal microflora. Expanding specific beneficial bacteria through diet-derived bacteria may have multiple health benefits, including the prevention of pathogenic bacterial growth, adhesion and penetration of mucosal surfaces; stimulation of mucosal barrier functions; and promoting an appropriate on the immune response. Recent probiotic research has focused on members of the genera *Lactobacillus, Bifidobacterium,* and *Streptococcus*. Although evidence for specific intakes needed to achieve health benefits is weak, consistent consumption is recommended to maintain probiotic-associated biological responses. Prebiotics are dietary substances, often complex polysaccharides, which support growth of probiotic bacteria *in vivo*. Human studies about the effects of probiotics and prebiotics on cancer risk are limited and inconsistent, but animal models provide strong evidence that these substances can prevent a variety of carcinogen-induced cancers. Further research is needed to rigorously assess the ability of specific prebiotics and probiotics to prevent cancer, including establishing effective doses and duration and frequency of consumption.

Commensal bacteria are essential for maintaining epithelial homeostasis and are involved in responding to pathogens. The effects of commensal and probiotic bacteria are mediated through the toll-like receptors (TLRs) and include cell survival, homeostasis, repair response, and inflammatory response. Commensal and probiotic bacteria provide TLR ligands, including lipopolysaccharide from Gram-negative bacteria, lipoteichoic acid from Gram-positive bacteria, flagellar proteins, DNA, and short chain fatty acids. These bacterial-derived ligands have been linked to the activation of gut-associated immune cells. *Lactobacillus rhamnosus* has been shown to modulate dendritic cell function, inducing peripheral hyporesponsiveness in stimulated CD4+ T cells. Probiotics may also affect NK cell activity. While NK cell activity is decreased in the elderly, consumption of probiotic bacteria by adults results in increased NK cell number,

ex vivo tumoricidal activity and increased ex vivo oxidative burst capacity of monocytes. In carcinogen-induced murine models, Lactobacillus casei Shirota (LcS) feeding delayed tumor onset, enhanced NK cell number cytotoxic activity due to LcS feeding. These findings suggest that enhancement of the cytotoxicity of NK cells by LcS delays tumor onset. These data establish the biological plausibility that commensal and probiotic bacteria may signal through epithelial cells, M cells (a specialized type of epithelial cell or dendritic cells to affect the activity of γδ T cells and NK cells. Because dendritic cells can directly sample luminal antigens, antigens derived from probiotic bacteria may be processed and presented by dendritic cells to mucosal immune cells. Recent data has established that reciprocal interactions, via direct contact and soluble mediators, between dendritic cells and NK cells can shape innate and adaptive immune responses. As such, the cancer preventive effects of probiotic bacteria may be mediated through immunomodulatory activities, including the antigen acquisition/presentation by dendritic cells followed by enhancement of NK cell tumoricidal activity.

Are Omega-3 Fatty Acids Effective in Enhancing Tumoricidal Cell Activity?

Kevin Fritsche, University of Missouri, Columbia

Many health claims have been made for omega-3 fatty acids, including reducing risk of death from myocardial infarction, lowering triglycerides, and reducing inflammation in conditions such as arthritis and irritable bowel disease. Weaker evidence exists for omega-3 fatty acids to reduce cancer risk; epidemiological studies of omega-3 fatty acids and cancer incidence in humans are complicated by recall bias and confounders. The influence on cancer incidence depends on whether the omega-3 fatty acid is from plants or marine sources. There is little evidence that omega-3 fatty acids reduce cancer incidence through their effects on the immune system, but they do appear to modulate the immune response.

Several studies have examined the effect of omega-3 fatty acids on components of the immune system. Macrophages from mice fed a diet high in omega-3 fatty acids showed increased tumoricidal activity *in vitro*. This was also associated with decreased PGE_2 production, which is normally stimulated by tumors and inhibits tumor killing. Other studies have shown that omega-3 fatty acids suppress lymphocyte proliferation in humans, rats, and mice; suppress cell-mediated cytotoxicity of macrophages, NK cells, and cytotoxic T lymphocytes (CTLs); and reduce antigen presentation *in vitro* and *in vivo*. Different omega-3 fatty acids appear to have different effects on the immune system, with omega-3 fatty acids such as menhaden fish oil, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) associated with a greater reduction in IL-12 and IFNγ production than olive oil.

NK cell activity has been found to be reduced in colitis patients fed a diet enriched in omega-3 fatty acids. In a rodent model, this effect was reversed by addition of IL-12. The number of NK cells was not changed, but tumoricidal activity was decreased; similar effects were observed for cytotoxic T lymphocytes. Enhanced growth and metastasis of C57Bl/6 mouse tumors was observed when these tumors were transplanted into mice fed diets high in omega-3 fatty acids, and cytotoxic T lymphocytes and macrophages in these mice had diminished tumoricidal activity. Omega-3 fatty acids may reduce tumoricidal activity by reducing biosynthesis of IL-12 and IFNγ, cytokines that promote Th1-type immune responses essential for generation of cytolytic CD8+ T cells and macrophages. Evidence implies that omega-3 fatty acids alter various activities of the immune system and reduce host tumor immunity.

Discussion

Dr. Cunningham-Rundles commented that although others have seen a difference in the effects of DHA and EPA, Dr. Fritsche's data did not show this. Dr. Fritsche responded that those studying omega-3 fatty acids need to analyze the differences in effects attributed to the various omega-3 fatty acids more carefully. Knowing the exact effects of specific omega-3 fatty acids within and without their food matrix will be valuable.

Dr. Anderson asked if IL-12 production from cultured monocytes could be inhibited by omega-3 fatty acids. Dr. Fritsche answered that this could be done *in vitro*, adding that dendritic cells and monocytes/macrophages both make IL-12. Omega-3 fatty acids do not, however, affect neutrophil production of IL-12. Dr. Anderson asked Dr. Fritsche to discuss possible mechanisms for this activity. Dr. Fritsche answered that the mechanism probably was not peroxisome proliferator-activated receptor γ-dependent and probably affects transcription rather than secretion of stores of IL-12. Dr. Milner added that questions concerning the effects of omega-3 fatty acids need to be considered when thinking about supplementing various foods with omega-3 fatty acids; particularly to vulnerable populations.

Are Fat-Soluble Vitamins Effective in Enhancing Tumoricidal Cell Activity?

Simin Meydani, Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University

The immune system, through a two-way interaction with tumors, is an important determinant of host resistance to cancer. Tumor prognosis depends on the ability of the host's immune cells to recognize and destroy tumors or resist tumor-induced suppression of the immune response. Tumors protect themselves from detection by the immune system through low expression of tumor specific antigens, lack of co-stimulatory molecules expression, antigenic mutation, production of suppressive factors, and growth surrounded by physical barriers. Nutrients, in particular fat soluble vitamins, may enhance host immune response to tumors by increasing tumor antigenicity, enhancing the effector function of innate and adaptive immunity and their interaction, and decreasing production of suppressive factors by tumors. Deficiencies in vitamins E and A are associated with diminished immune response in several compartments of the immune system. Proliferation and function of T cells and NK cells is decreased, as is production of several cytokines important for tumoricidal activity. There is little evidence that increasing amounts of vitamin A above RDA has an effect on the immune system, but supplementation with vitamin E above RDA levels improves T cell function and NK cell activity, as well as decreases PGE_2 production. Most of the research related to vitamin A and E and immune response has been conducted in non-tumor bearing host, and thus might not be reproducible in tumor bearing hosts. Some of these findings, particularly those conducted in the aged might be useful in relation to the role nutrient/immune system interaction in cancer prevention.

Cancer incidence and mortality increase with age, caused in part by declines in immune system function. Macrophages of aged have increased production of PGE2 and free radicals, resulting in inflammation and T cell suppression. Decreases in cytotoxic T lymphocytes activity, Th1/Th2 cells, T cell proliferation and IL-2 production, and DTH reactions are also observed. Supplementing aged mice and elderly people with vitamin E increases T cell function; optimal vitamin E dose for improving the immune response in the elderly is 200 International Units. Two possible mechanisms for this improvement

include inhibition of PGE_2 production by macrophages or directly affecting the ability of naïve T cells to divide and produce IL-2.

T cells isolated from the spleen of young (4 to 6 months of age) and old (22 to 26 months) mice that consumed vitamin E (RRR-α-tocopherol) were activated with anti-CD3 and anti-CD28 monoclonal antibodies. Cells from older mice had decreased rates of proliferation and IL-2 production compared to younger mice. Exposure to vitamin E increased proliferation and IL-2 production in cells from old mice, but not in cells from young mice. The ability of vitamin E to increase IL-2 production was caused by increasing the number of IL-2-producing T cells and increasing the amounts of IL-2 made by these cells. Vitamin E increased the capacity of T cells from old mice to divide; this was specific to naïve cells. Several experiments examined T cell signaling mechanisms to determine how vitamin E affected T cell division. Confocal microscopy was used to visualize immune synapses, which are areas of conjunction between T cells and antigen-presenting cells. Synapse formation was decreased in cells from older animals, but vitamin E increased the percentage of effective immune synapses formed in these cells. Staining for specific signaling molecules (Vav, Zap-70 and LAT) showed that supplementation of old mice with 500 parts per million (ppm) vitamin E increased redistribution of these molecules to immune synapses. Vitamin E also was observed to increase NK cell activity in older mice. The ability of vitamin E to improve immune response to infection also was described. Young and old mice were fed 500ppm vitamin E for 30 days and then challenged with influenza virus. A greater decrease in influenza virus titer was seen in older animals supplemented with vitamin E. These animals also had increased production of IL-2 and IFN-γ, which was associated with decreased virus titers.

Future anti-tumor therapies must induce and sustain activity and survival of cytotoxic T lymphocytes and other anti-tumor cells, optimize lymphocyte functions in the tumor microenvironment, and prevent immune suppression by inhibiting production or activity of tumor-derived suppressive factors and inhibiting generation or function of CD4+ CD25+ regulatory cells. Determining the efficacy of fat soluble vitamins to improve tumor-specific immunity in both normal and immunodeficient hosts must address all aspects of immune response to tumors, including tumor antigenicity, reduction of tumor-derived suppressor factors, and improvement of tumor-specific innate and adaptive immunity.

Discussion

Dr. Erdman asked which forms of vitamin E were key to improved immune synapse formation. Dr. Meydani answered that we have not compared the effect of different tocopherols on immune synapse formation specifically, but all tocopherols have the ability to improve T cell proliferation, but the effective dosage varies. γ, δ, and β-tocopherol are effective at lower dosage than alpha-tocopherol; however, they can be more toxic. The dosage at which α-tocopherol improves immune system function is actually a cytotoxic dose for the other tocopherols. Dr. Milner asked whether the effect was due to a tocopherol phosphate or other metabolites of tocopherol. Dr. Meydani answered that experiments are planned to determine whether this is a direct effect or mediated through metabolites. Dr. Milner commented that tocopherol phosphate could mediate this effect through cellular transcription. Dr. Meydani answered that this was true; the effect could be due to other factors involved with signaling molecules located at the cell membrane.

Are Botanical Glucans Effective in Enhancing Host Immune Response to Tumors?

Susanna Cunningham-Rundles, The Weill Medical College, Cornell University

Mushroom polysaccharides, particularly beta-glucans, have anti-tumor and immunostimulating properties. These polysaccharides do not directly attack cancer cells, but instead exert their effects by activating immune responses in the host. Betafectin PGG-glucan enhances hematopoietic recovery in immune-suppressed mice and primates by synergizing with myeloid growth factors. Another beta-glucan, soluble branched (1,4)-beta-D-glucan, augments NK activity and cytotoxic T lymphocytes response to enhance anti-tumor activities against MHC class I-negative and -positive malignant melanoma. Use of beta-glucans as adjuvants for monoclonal antibody treatment has also been explored, because beta-glucans bind to the complement receptor 3 (iC3B) and could enhance leukocyte killing of tumor cells coated with iC3b through naturally occurring anti-tumor antibodies. Beta-glucans also appear to recruit tumoricidal granulocytes, dramatically enhancing tumor killing when combined with monoclonal antibody therapy. Barley beta-glucans enhance anti-tumor effects of monoclonal antibodies against human tumors transplanted into SCID mice, decreasing the size of the tumor more rapidly than antibodies alone. A clinical trial using this beta-glucan as adjuvant therapy in children with neuroblastoma showed that it may be beneficial in humans. Mushroom beta-glucans may affect immune function by infiltrating tumors and activating dendritic cells, suppressing certain P450 enzymes, or by influencing mitotic activity. Despite promising initial results, a significant obstacle for this research is the difficulty in obtaining purified and reliable forms of mushroom polysaccharides.

The active component of the MD-fraction (MDF) of Maitake mushrooms is 1, 3-branched 1, 6-glucan. This mushroom has been traditionally used to treat breast and other tumors in Asia. When given to children receiving chemotherapy, Maitake mushroom lessened their need for blood support products. For studies described here, MDF was obtained from mushrooms grown hydroponically in New York. To determine the effects of MDF on the immune system, mouse bone marrow cells (BMC) were exposed to MDF and assayed using a colony-forming assay (detection of colony forming unit granulocyte macrophages). Colony-forming activity of BMC was increased in a dose-related manner, with the most activity observed at 100 µg/ml, followed by a drop in colony forming activity at 200 and 500 µg/ml. The increased activity appears to be due to enhanced viability of murine BMC upon treatment with MDF. MDF also protected BMC from doxorubicin toxicity, again through protecting BMC viability. MDF also was observed to promote recovery of BMC from doxorubicin-induced cytoxicity.

Similar experiments were performed using human umbilical cord blood cells. MDF also enhanced colony-forming activity in a dose-dependent manner, with inhibition of this activity seen at MDF doses greater than 100 µg/ml. MDF also protected cord blood cells from doxorubicin toxicity by affecting early viability. MDF partly replaces granulocyte colony stimulating factor (G-CSF) in colony formation system and enhances G-CSF production from CD33+ cells. MDF also stimulates TNF-α, IL-10, and IL-1β production by U937 cells treated with phorbol myristate acetate. Dose response studies are hindered by differences in activity in different batches of MDF, with colony-forming activity varying greatly over the different batches.

Beta glucans promote hematopoietic bone marrow cell viability and promote protection against doxorubicin toxicity and therefore may be an excellent adjuvant to cancer

therapy. Consumption of these carbohydrates may enhance innate immunity and result in cancer prevention.

Discussion

Dr. Daniel Silva asked whether Dr. Cunningham-Rundles had information on signaling pathways affected by MDF and whether these were specific to certain cell types. She answered that they had not observed effects on cell cycle, and do not think that the extract will directly affect tumor cells, cell cycle, or cytokine production.

Dr. Milner asked if she had examined other sources and types of mushrooms. Dr. Cunningham-Rundles responded that the source of the mushrooms may be important to mushroom activity, given differences in soils in which the mushrooms are grown. Her group will be experimenting with different mushrooms to see if they have similar activity.

Is Glutamine Effective in Enhancing Tumoricidal Cell Activity in a Breast Cancer Model?

V. Suzanne Klimberg, University of Arkansas for Medical Sciences

Glutamine (GLN) is necessary for *in vitro* growth and function of T lymphocytes and NK cells. Glutathione (GSH) is an important antioxidant and plays a key role in defense and cell signaling. Tumor cells act as GLN traps, leading to net decreases in available GLN and GSH. *In vitro* assays have shown that GLN stimulates tumor growth, but *in vivo*, oral GLN suppresses tumor growth in implantable tumors and chemically-induced models of carcinogenesis. Supplementation with GLN is associated with enhanced NK cell activity and decreased tumor volume. Under normal conditions, supplemental GLN increases arterial GSH by 40 percent and enhances GSH gut release 3-fold. PGE_2, which is produced by tumors and down-regulates NK activity, is decreased by GSH. Oral GLN decreased tumor growth by 50 percent in an implantable tumor model and this decrease was associated with increased arterial and gut GSH concentration and increased GSH release. Oral GLN also increased NK activity in normal patients and increased NK activity in cancer patients by an even greater amount.

The ability of GLN to affect carcinogenesis by influencing initiation or promotion was explored using a chemically-induced rat mammary carcinoma model (7, 12 dimethylbenz [a] anthracene [DMBA]). Oral administration of GLN, starting 1 week before animals received DMBA up to sacrifice (11 weeks), reduced breast tumor incidence and increased NK cell activity compared to rats receiving water or isonitrogenous Freamine®. Tumors arising in GLN-supplemented rats often were non-malignant and tumor size and weight was reduced. Arterial GLN concentrations were elevated and arterial GSH concentration was normal in animals receiving GLN; arterial GSH was decreased in animals receiving Freamine® or water. Supplemental GLN also restored normal GSH and GLN gut extraction by 1 week after supplementation began. Oral GLN increased intracellular GSH in mammary cells, opposing the action of DMBA, which decreases GSH in mammary cells.

Studies comparing long-term (11 weeks) to short-term (4 weeks) GLN supplementation suggested that both treatments reduced tumor number, implying that the first 4 weeks after DMBA administration are critical for GLN supplementation. GLN supplementation and its accompanying elevation of blood and tissue concentrations of GSH are also associated with decreases in serum insulin-like growth factor-1 (IGF-1) and TGF-β levels. High concentrations of IGF-1 are associated with increased PGE2 levels and

increased risk of several human cancers. Thus, down-regulation of IGF-1 may be one way GLN mitigates tumorigenesis.

Supplemental GLN may prevent tumor growth through activation of redox-dependent signaling. This hypothesis is based on the observations that GLN prevents tumor growth in implantable as well as DMBA-induced breast and squamous cell cancer models; GLN supplementation inhibits the production of natural anti-oxidant glutathione (GSH) in tumors while enhancing it in normal tissues; and GLN activates p53 signaling and inhibits PI3K/Akt signaling in a DMBA-breast cancer model.

Discussion

Dr. Fraker asked whether tumors dividing at a reasonable metabolic rate take up GLN. Dr. Klimberg answered that they do, and this is accompanied by a decrease in gene expression of enzymes and factors including glutaminase activities involved in GLN cycling in tumors.

Dr. Milner asked whether release of ammonia from glutamine is a factor. If glutamic acid is compared to GLN, is a similar type of effect observed? Dr. Klimberg answered that she does not see an effect using ammonia. If increased GLN concentration is important, expectations are that a person would be better served with a high rather than a low protein diet. Dr. Klimberg answered that she believes the intermediary is GSH because that is what seems to be affected, along with PGE2. Dr. Milner asked about proper controls for this work, because general amino acid catabolism can ultimately increase cellular GLN concentrations. Dr. Klimberg answered that her control was to use a mixture of amino acids, such as Freamine.

Dr. Jeffery asked whether Dr. Klimberg had assayed plasma GSH as well as cellular GSH. Dr. Klimberg answered that she had looked at whole blood because red blood cells have high amounts of GLN. Dr. Jeffery commented that this implies that Dr. Klimberg had looked primarily at cellular rather than extracellular GLN. Dr. Klimberg answered that this was true; she had primarily examined cellular GLN. She added that the data showed that total GSH was reduced. She also analyzed the ratio of oxidized to reduced GSH. Dr. Jeffery asked if Dr. Klimberg could add in something that would affect GSH. Dr. Klimberg answered that glycine, which was used in the studies, also affected GSH. Dr. Jeffery asked if there was something more specific to GSH that could be used. Dr. Klimberg answered that people have used n-acetylcysteine, which will increase GSH levels. Her group is also attempting to chlorinate GLN so that it only makes GSH.

Summary and Discussion

Susan Percival, NSRG, DCP, NCI

Dr. Percival thanked the attendees for their participation. She noted that several participants had indicated that a greater understanding about the tumor's interaction with immune cells was needed to develop better strategies for cancer treatment and prevention. While there is no direct evidence exists that nutritionally induced changes in immunity lead to prevention of cancer risk or tumor behavior, there is evidence that diet can influence various parts of the immune system. She noted that both animal models and cell culture studies indicate that tumoricidal cell activities can be enhanced by several bioactive food components. Since tumoricidal cell activity is necessary for immunosurveillance; it is assumed but not documented that do not have the direct

evidence this change influences cancer prevention. Regardless, there is no doubt, that nutrition plays a critical role in overall immune function.

Overall, little is known about the basic biology of γδ T-cells and NK cells in deterring cancer or in eliminating aberrant cells, in general. Nevertheless, γδ T-cells have the potential to destroy epithelial cancer since of their general location. NK cells may be more effective at retarding cancers throughout since they tend to migrate throughout the body. Regardless, more research is needed to define which cell is more effective and under what circumstances.

Attendees indicated that a greater understand of γδ T cells was a critical area that needs to be studied since about 80% of all immune cells are in epithelial tissue and in contact with the outside environment, yet the majority of all immunologists study the immune cells in the other tissues/blood (about 20%). Areas of specific study include: What types of antigen do γδT cells "see"? Can we use these antigens to turn on the γδ T cells and fight cancer? How do γδT cells get turned on? If γδT cells have negative effects, how do we turn them off?

Bioactive food components likely have different influences on NK and γδ T cells. It is unclear why differences exist and if common mechanisms are involved. For example, it is sometimes difficult to determine if the response is as a result of being an antioxidant or via some other mechanism. A concern was raised that by potentially lowering cancer risk by the use of pharmacologic amounts of bioactive food components might in some circumstances create unwanted consequences. In other words, cancer risk may be reduced, but risk for other diseases may be increased; for example, over-stimulated γδT cells might increase inflammatory bowel disease. It was also pointed out that pharmacologic supplements may not be necessary to bring about a response. Thus additional research is needed to clarify appropriate quantities and specificities among bioactive food components that are needed to bring about a beneficial tumoricidal response.

Undeniably, immunity and cancer incidence can be modified by diet. However, it is unclear if these are directly or indirectly linked. Adoptive transfer models in which lymphocytes from one animal can impact tumor progression in another provide valuable information about the diet-immunity interrelationship. Other models are likely needed to adequately evaluate tumoricidal activity as affected by diet, especially that which can assist in predicting cancer outcomes.

Dr. Tibiani commented that researchers must consider the micro-environments in which foods are grown, including season of harvest, which can affect alkyloid composition as seen for Dr. Cunningham-Rundles' mushrooms or the L-theanine content of tea. Researchers also should be specific about various forms of a bioactive food component, for example, long-chain versus short-chain beta-glucans or the use of EPA versus DHA versus fish oil.

Dr. Tibiani again mentioned NCI's RAPID (http://programs-resources/programs/rapid) and RAID (http://nihroadmap.nih.gov/raid/) which may help investigators standardize bioactive food components. Dr. Milner added that there is an RFP that supports applications to examine standardization of bioactive components and how the matrix may modify cancer risk (OMB No. 0990-0115; http://grants.nih.gov/grants/funding/sbir.htm).

Dr. Fraker commented that Drs. Bukowski and Meydani presented interesting studies that begins to describe likely mechanisms by which food component may alter the

immunity. She noted that additional studies of this sort are needed to clarify appropriate recommendations to individuals. It was also indicated that difficulty may occur in obtain significant numbers of the γδ T cells or the NK cells for ex vivo studies (i.e. western blotting, biochemical assays of all sorts). Therefore, transgenic and knockout models may be very useful in sorting through mechanisms.

Dr. Klimberg commented that although some clinical trials have large study populations, it may be difficult to conduct studies adequately to examine the cancer-preventive effects of dietary components. Validated surrogate markers, such as NK cell activity or IGF-1 levels, could be useful to help clarify the specific response.

A participant asked which populations would be best to study. The elderly sometimes have a breakdown in immune surveillance, increased cancer incidence, and increased nutritional deficiencies. If a study seeks to examine the use of nutritional supplements as adjuvants to immune-based therapies, it might be best to examine the elderly population. Dr. Bukowski agreed that clinical studies in at-risk populations, perhaps studies that try to prevent recurrence, would be most useful. Carefully chosen at-risk populations could allow the use of smaller populations. One comment made that in addition to the elderly and military personnel might also be an appropriate population to study because of increased stress.

Dr. Anderson thought that nutritional interventions for cancer treatment might have a lower chance of success, because tumors frequently evolve strategies to evade these interventions. He suggested a leukemia-susceptible mice model might be used to determine the ability of nutritional interventions to reduce/prevent cancer recurrence. Dr. Milner added that some bioactive food components have the same sites of action as some drugs and therefore provide comparable protection. Thus, several models might be useful in examining the tumoricidal properties of food components. If the site of action of these components could be identified, this would be key to identifying a subset of the population who would respond to intervention. Dr. Anderson agreed that perhaps combination therapy with bioactive components and drugs could be used to treat an existing tumor.

Dr. Jeffrey noted that there may not be sufficient funds for large trials and thus short term studies with selected biomarkers may be a viable alternative to promoting research dealing with tumoricidal properties of food components. A solution may also be to piggyback onto existing trials. Good biomarkers will be essential to progress in this area. It was noted that scientists must consider the timing and duration of exposures in evaluating outcomes. Since research on diet and tumoricidal activity will encompass broad areas within cell biology, nutrition and immunology, a special emphasis (review) panel may be needed to assist with the evaluation of this cross-cutting area.

Another key point was issues surrounding appropriate biological markers. It remains unclear how best to monitor cancer risk reduction in short-term studies. The fundamental question remains "Do increased immune cell numbers mean anything?" Likewise, it remains to be resolved what immune markers are best correlated with a good clinical outcome, i.e., cancer prevention.

At-risk populations in which cancer may reoccur may be very useful for examining the effects of bioactive food components. However, the disadvantage of these populations is that individuals who have already had cancer may not represent risk in other populations. Again, it was noted that elders may be a particularly vulnerable group because they frequently have immune dysregulation, increased risk for nutrient deficiency, and increased risk for cancer. Dr. Seifried commented that prevention is

likely to be an early event and thus attention to older individuals may result in misinformation about the relevance of the diet. Even when susceptible populations are used, it may be necessary for trials to continue for long periods to determine a true response. Dr. Klimberg pointed out that tamoxifen trials to determine the ability of this compound to prevent breast cancer in the opposite breast had been successful and thus target population can be used for some types of studies. She added that endpoints could also be employed to determine early events; perhaps using dysplasia rather than cancer. She also advised researchers to keep sample collection simple, because if it is frequently difficult to collect multiple samples from subjects, and might even jeopardize the success of the trial.

It was again noted that γδ T and NK cells represent a vital branch of immunity involved in immunosurveillance and likely involved with cancer prevention. Exciting opportunities are ahead to understand mechanisms of action, their basic biology and useful biomarkers that are both comprehensive in methodological approach and specific to the mechanism by which these cells kill cancer cells. While challenges exist to design studies that give direct evidence rather than indirect associations, to recognize who represents a vulnerable population, and to be complete enough to show enhancing one branch of immunity does not have detrimental effects on other branches, there are numerous opportunities for advances in this area. The interaction of nutrition, immunity and cancer prevention is undeniably emerging as an increasingly important field of study. The future holds great promise that cancer can be prevented or reduced by changing the activity of γδ T and NK cells through personalized nutrition.