Meeting Report and Abstracts

Report and Abstracts on the Workshop on “Plant Cell Wall Polysaccharides and Their Impact on Human Health”

(J-STAGE Advance Published Date: July 5, 2013)

REPORT

Supported by the Japanese Society of Applied Glycoscience, Japanese Society of Food Hydrocolloids, Japan Association for Food Function Clinical Research, and University of California, a workshop, entitled: "Plant Cell-Wall Polysaccharides and Their Impact on Human Health", was held at the University of California, Davis Conference Center on October 5, 2012, with a total of 7 (2 keynote and 5 public) lectures covering a broad range of topics on cell-wall polysaccharides, from plant physiology, polysaccharide chemistry, and immunology to the clinical applications of cell-wall polysaccharides as functional fibers and the current status of health claims in the United States. The key concept of this workshop was to gain further insights into plant cell-wall polysaccharides (which are also classified as dietary fibers) from different perspectives, making the workshop an unprecedented, unique event. Each lecture was followed by an active exchange of comments and feedback, and was received with great interest from a total of nearly 50 participants. At the dinner banquet, which was held after the lecture session at the on-campus Hotel Hyatt Place, the participants had a good opportunity to promote friendships and networks with each other as well as to celebrate Dr. Nevins’s upcoming retirement from the University of California. This workshop was first planned by Drs. Shinichi Kitamura (Professor, Osaka Prefecture University), Hiroaki Maeda (President, Origin Biochemical Laboratory, Inc.) and Yoji Kato (Vice president, Hirosaki University) during their conversation on the topic of “plant cell-wall polysaccharides and their physiological roles” at an annual meeting of the Japanese Society of Applied Glycoscience held in Sapporo in September 2011. Thereafter, an executive committee was formed including a group of experts in the relevant fields who agreed to the project, and the workshop was successfully held at the University of California, Davis. Despite being only a one-day event, this workshop was very productive and fruitful.

Yoji Kato
Hirosaki University
(Chair of the workshop)
Keynote lecture by Dr. Schneeman

Speech by Dr. Nevins at the dinner banquet

An active exchange of comments

Workshop participants
Workshop on "Plant Cell Wall Polysaccharides & Their Impact on Human Health"

PROGRAM

09:00 - 09:20  Yoji Kato Ph.D. (Hirosaki University)
Opening address

PL 1  09:20 - 10:20  Donald Nevins Ph.D. (University of California, Davis)
A personal perspective of how changes in polysaccharide analytical methods give insights into functions

10:20 - 10:40  Coffee break

IL 1  10:40 - 11:20  Naoki Sakurai Ph.D. (Hiroshima University)
Functions of polysaccharides of the plant cell walls

IL 2  11:20 - 12:00  Kenji Tazawa M.D., Ph.D.
(Toyama Medical and Pharmaceutical University)
Portal blood purification and apple pectin: How apple pectin inhibits liver metastasis of cancer

12:00 - 13:30  Lunch

PL 2  13:30 - 14:30  Barbara Schneeman Ph.D.
(The Food and Drug Administration)
Dietary fiber: Current policy and future directions

IL 3  14:30 - 15:10  Hiroshi Tsunekawa M.D., Ph.D. (Tsunekawa Clinic)
Hydrolized hemicellulose from purple rice prolongs survival in cancer patients

15:10 - 15:30  Coffee break

IL 4  15:30 - 16:10  Kiyoshi Hayashi Ph.D.
(National Agriculture and Food Research Organization, JP)
Research on elucidation of functions of agricultural products and foods in NARO & NFRI

IL 5  16:10 - 16:50  Noriko Tsuji Ph.D.
(National Institute of Advanced Industrial Science and Technology, JP)
Particulate β-glucan induces anti-inflammatory CD4+ regulatory T cells in Payer’s patches

16:50 - 17:30  Discussion

17:30 - 17:45  Ryoichi Obitsu M.D., Ph.D. (Obitsu Sankei Hospital)
Concluding remarks
Using a unique previously uncharacterized xylanase we found suppression by certain bran preparations. And has been exploited extensively as a basic procedure for plant wall analysis. In our subsequent work we focused on the walls of grass plants. Early on we found that during development there was significant turnover in the wall of a noncellulosic glucan based on compositional analysis. We concluded the glucan was not the result of starch contamination because we pretreated wall preparations with a porcine α-amylase. But others repeating the experiments could not confirm the presence of the glucan in similar wall samples. The only difference in our two approaches was the source of the amylase. The other group had used 4x crystalized Bacillus subtilis amylase. To resolve the discrepancy we subjected the Bacillus amylase and the porcine amylase to extensive purification. Profiles showed a single protein with amylase associated. However in fractions clearly separated from the Bacillus amylase was another enzymic activity localized in the elution profile but not associated with any detectable protein. That activity was shown to be β-(1-3)(1-4)-glucanase an enzyme known to be produced by Bacillus. Hence the report of the absence of a glucan by the other research group was erroneous because the amylase used was contaminated and specifically removed the noncellulosic glucan. The results demonstrated the need for stringent evaluation and characterization of enzymes applied in analytical procedures. We all became sensitive to errors generated by possible contamination in enzymes. However the fact that the enzyme was so specific for the glucan offered a powerful analytical tool for extracting and characterizing a particular polysaccharide in wall preparations. And has been a key diagnostic in the analysis of the glucan responsible for cholesterol suppression by certain bran preparations.

Xylans are other major components of the grass cell wall. Using a unique previously uncharacterized xylanase we were also able to characterize an arabinobioxylan of primary walls that was distinct from other grass xylans. The enzyme cleaved xylans into discrete fragments via a motif recognition. The action pattern of this xylanase had not been previously described. As a part of the grass xylan characterization we found the attachment point for ferulic acid. Ferulic acid attached to polysaccharides suppresses digestibility in animal feed. But the ferulic acid attached to xylan also serves and important function in the grass wall because it represents the anchor for lignins to polysaccharides.

It is becoming increasingly clear that digestive processes in animals is facilitated by enzymes generated by an interaction with a complement of intestinal microbes. The coexistence of microbes in the human digestive system offers nutritional benefit and the complex of interactions is currently being resolved and is of great interest to the medical community (J.K. Nicholson et al.: Host gut microbiota metabolic interactions. Science, 336, 1262-1267 (2012)). From the stand point of cell wall polysaccharide analysis it is likely that a continuing array of analytical tools will be identified as the features of microbial polysaccharidases are resolved.

***

Functions of Polysaccharides of the Plant Cell Walls

Naoki Sakurai1,*

1 Hiroshima University
(1-7-1 Kagamiyama, Higashi-Hiroshima 739-8521, Japan)

INTRODUCTION

Terrestrial plant is regarded to stand against gravity by its cell walls, although it lacks bones made of calcium, which most of terrestrial animals have. Vegetables or fruit are not sustained or hold its shape only by the function of the cell walls. For example, immature banana is very hard enough to repel blade of knife, but once treated with gaseous plant hormone, ethylene, it quickly becomes soft. During this softening process, the cell walls become soft, but degradation of starch grain within the cells mostly contributes to the softening of banana. Vegetables become wilting after harvest, when stored for long time under ambient dry condition. After wilting, the cell walls still remain, but loss of turgor (potential force for sucking water from outside of the cell) causes wilting of vegetables. Plant can not retain its sharp without turgor even with cell walls.

One doesn’t notice an unbelievable increase in the volume of plant cells, especially since tree grows slowly, but plant increases the height mostly by its elongation of each individual cell. This elongation process is one of the specific characteristics of plant, which animal lack. Tomato or corn, when sown in spring or early summer, sometime grows taller than us at the end of summer. The cell size of these plants longitudinally increases more than ten times (Fig. 1).

This increase in cell size is designated as an absorption growth, since cell absorbs water during its growth. Water absorption results from the higher osmotic pressure of the cytoplasmic solution within the cells than that of outside of...
Workshop on "Plant Cell Wall Polysaccharides & Their Impact on Human Health"

Fig. 1. Changes in cell volume during cell elongation growth.

Plant cell increases its volume about ten times during cell elongation growth. This process mainly consists of water absorption. Well elongated cell develops a big vacuole.

the cells. Such longitudinal increase in the cell size is designated as elongation growth. For the elongation growth, area of the cell walls must increase or at least retain its extensibility (high potential to increase its surface). Hard cell wall architecture does not allow elongation growth. Cell walls of young plant that have high potential to grow are not only mechanically tough but also flexible or plant. When fruit becomes ripe, fruit shows soft texture. It is well known that during this ripening process of fruit, the cell wall composition substantially altered.

Cell walls of plant that retains high potential to grow and is actively growing (increasing cell volume) are designated as primary wall. Cell walls constructed after the growth ceases is designated as secondary wall. The primary wall consists of acidic polysaccharides (pectin), neutral polysaccharides (hemicelluloses) and cellulose at the ratio of 1:1:1. The secondary wall consists mostly of cellulose and orientation of cellulose microfibrils is random. The microfibril orientation of primary cell walls is arranged horizontally, such as hoops of a barrel. Then the direction of plant growth, especially stem, is acropetally oriented to sky and plant develops leaves to absorb solar energy, because of limitation of lateral expansion by horizontally oriented cellulose microfibrils. When the growth ceased, orientation of cellulose microfibrils becomes random, leading to suppress further acropetal growth.


Cellulose microfibrils have very high tensile strength, because 1,4-β-glucan chains are bonded each other by many hydrogen bonds and exhibit crystalline fibril structure. It has long been hypothesized that the highly extensible cell wall property of the primary wall attributes to matrix polysaccharides embedded between two adjacent microfibrils. First, calcium cross linking of pectic polysaccharides was the candidate for the endowment of high extensibility of the primary cell walls, but later the hemicellulosic polysaccharides attracted more attention.

Cell walls of monocotyledonous grass plant have little pectin, while those of dicotyledonous plant are rich in pectin. Therefore, hemicellulose has been considered responsible for the cell wall extensibility in grasses plant. Hemicellulosic polysaccharides of grasses cell walls are extracted by strong alkaline solution. The extracted materials consists of two main hemicellulosic polysaccharides, glucuronoarabinoxylans and 1,3;1,4-β-glucans (Fig. 2). There is almost little amount of 1,3;1,4-β-glucans remained in grass culm or stem after anthesis, remaining glucuron(arabin)oxylan with trimmed arabinose residues that had been attached to backbone xylan chain in younger stage. This fact implies that degradation of 1,3;1,4-β-glucan is associated with growth of grass plant. Loescher and Nevins (1972) reported that treatment of grass plant tissues with auxin (plant growth hormone) reduced amount of 1,3;1,4-β-glucan in the cell walls when auxin induced elongation growth. This decrease caused by auxin was not affected by high osmotic concentration of ambient test solution that inhibited water-absorption growth. It suggested that the degradation of 1,3;1,4-β-glucan in the grass cell walls was responsible for the auxin-induced elongation growth.

2. Properties of 1,3;1,4-β-glucan in the cell walls of grass plant.

Molecular weight of 1,3;1,4-β-glucan of cereal plant is over one million, being very long polysaccharide polymer. Consecutive long 1,4-β-linked glucose residues are lacking and one 1,3-β-linked glucose residue is interpolated every two or three 1,4-β-linked glucose residues (Fig. 2). There is scarcely found crystalline domain in the molecules, simply because the above described structural feature of 1,3;1,4-β-glucan prevents inter- and intra-hydrogen bonding in the 1,3;1,4-β-glucan molecules. This lacking of inter- and intra-molecular hydrogen bonds allows the molecules soluble in water, but the solution is highly viscous, because of its high molecular weight. It was assumed, therefore, that the high molecular weight of this glucan afforded viscous nature to the cell walls in muro. For example, when the molecular weight is high, the cell walls are hard, while the cell walls become softer when it degrades into smaller molecules.

The 1,3;1,4-β-glucan is hydrolyzed by two types of enzyme, exo- and endo-1,3;1,4-β-glucanase. Bacterium, Bacillus subtilis, is actually producing these two types of enzymes to hydrolyze cereal 1,3;1,4-β-glucan. This bacterium is frequently present on the surface of cereal straw. It was assumed that if plant cell secretes these types of glucanases into the cell walls, then the regulation of the enzyme activity in the cell walls is closely involved in the cell elongation.
growth. Finally, two types of enzyme activities were found in the cell walls of barley plant. Study of amino acid sequence of the enzymes led to the finding of exo- and endo-glucanase genes, leading to the expression experiment of these genes in the presence or absence of auxin.

Expression study of these genes revealed that exo-type glucanase genes were constitutively expressed irrespective of auxin treatment, while endo-type glucanase gene was expressed only in response to auxin treatment. It seemed quite reasonable, because the expression of endo-type glucanase gene is substantially more effective and specific in degrading 1,3;1,4-β-glucan than that of exo-type gene. There, however, was one unsolved and awkward problem, time lag of the gene expression.

Auxin induces elongation growth of plant cells within 1 h, while gene expression of endo-glucanase was detected four hours after auxin treatment. If the degradation of the glucan is truly responsible for auxin-induced growth, the gene expression should be detected ideally before auxin-induced growth or at least, as soon as auxin induces elongation growth. Osmotic control experiment was expected to solve this discrepancy.

3. Osmotic control experiment.

Generally, plant physiology uses mannitol to regulate osmotic condition of the plant cells. Mannitol has been used as an ideal osmoticum to control osmotic condition of the plant cells. It is very soluble in water, and expected not to transport into the inside of plant cells, and non-toxic. One can create the condition that the cell wall is changed to be extensible by auxin, but auxin doesn’t cause any apparent elongation (Fig. 3). In Figure 3, at the ambient osmotic concentration of 0.3 M mannitol, the osmotic concentration of the cytoplasm of the plant tissue is equivalent to that of the ambient solution. The plant cells neither absorb water nor increase the cell volume. Even under such condition, the cell walls are loosened by the action of auxin. When the sections that had been treated with 0.3 M mannitol are transferred to solution without any osmoticum and auxin, drastic induction of elongation of the segments takes place, proving the action of auxin on loosening cell walls even under the osmotically restricted elongating condition. If we found that endo-type glucanase gene is expressed by auxin action even under such condition where auxin-induced elongation was restricted, this finding might be a clear evidence that endo-type glucanase is responsible for auxin-induced elongation growth. But, repeated experiments showed vague results. Finally, sucrose was used as an osmoticum, replacing with mannitol. The results are unexpected but solved the problem.

When we used high concentration of sucrose, it suppressed auxin-induced elongation very effectively and curiously the gene expression of endo-type glucanase as well. Therefore, we reduced step-by-step concentrations of sucrose in the experiment. Very low concentration of sucrose that doesn’t inhibit any auxin-induced elongation, rather promote a little bit elongation, completely nullified the gene expression. The close correlation found between auxin-induced elongation and 1,3;1,4-β-glucan degradation was first broken. Replacement of sucrose with glucose also gave the same result. What this result means is very clear.

4. Function of 1,3;1,4-β-glucan present in the cell walls of cereal plants.

Plant physiologists have been using a strange plant tissue for their growth experiments. It is a coleoptile of grass plant. It is somehow a heritage of Darwin’s pioneer work of phototropism. He used coleoptile tissues of grass plant in his phototropism experiment with his son, Francis. Coleoptile tissue has an advantage, since this tissue grown only under the soil is sensitive to light. Coleoptile, a cylindrical tissue, develops from cereal seeds, in which first leaf is developing. Under the soil, coleoptile grows until it emerges from the soil. In other word, light stops its growth. Therefore, coleoptile function is to prevent young, soft leaf from the friction of rough soil particles and support them to reach the ground level. In fact once coleoptile reached ground level, it stops growing and leaf emerges from a small hole at the tip of coleoptile. Coleoptile, therefore, is an organ that basically grows under darkness in the soil. It is reasonable that there is almost no chlorophyll in the coleoptile tissue. During the growth underground, the energy or carbon source is delivered only from the seed. Plant physiologists have been using segments excised from the coleoptile and treating the segments with auxin in the elongation experiments. Coleoptile segments excised from the seedlings is disconnected to the supplier of carbon source, seeds. Therefore, the segments are facing to starvation, when incubated in the test solution unless artificial carbon source is added to the solution.

When such starved coleoptile segments are treated with auxin and increase their volume, new cell walls and cell membrane should also be synthesized. These syntheses need a plenty of energy. How do starved coleoptiles obtain the energy or carbon source for elongation? The gene expression experiment where small amount of sucrose or glucose nullified endo-type glucanase gene expression clearly revealed that carbon source is 1,3;1,4-β-glucan within the
Workshop on "Plant Cell Wall Polysaccharides & Their Impact on Human Health"

---

cell walls.

The 1,3,1,4-β-glucan is food for emergency. That is why the presence of small amount of sugar outside of the cells reduced the gene expression to degrade 1,3;1,4-β-glucan. Coleoptile segments primarily utilize carbon source already present in the ambient solution to save energy for gene expression and protein synthesis. In fact, Loescher and Nevin (1974) found that when the coleoptile segments were kept in water solution for several hours before auxin treatment, the effect of auxin on the decrease in 1,3;1,4-β-glucan content was more prominent. The same starvation experiment also caused stronger expression of the endo-type glucanase gene. These results strongly suggest that the higher the starvation level, the stronger the degradation of 1,3;1,4-β-glucan. These results also clarified that the degradation of 1,3;1,4-β-glucan that had been regarded as a key chemical change in wall components for cell wall extensibility and cell elongation, is not directly associated with auxin-induced elongation or a change in mechanical properties of the cell walls (Fig. 4).

5. Cell walls of dicotyledonous plants.

In dicot plants, xyloglucans (Fig. 5) are thought to be degraded in response to auxin and involved in auxin-induced elongation growth. Xyloglucans are consisted of 1,4-β-linked glucan backbones and xylose, galactose and fucose as side chain residues. Curiously enough, this polysaccharide was first found as a carbon source polysaccharide stored in the bean seed. It degrades, when the seed germinates. There is no evidence to deny the possibility that the xyloglucan degradation in response to auxin also functions as a carbon source, as well as 1,3;1,4-β-glucan.

Is 1,3,1,4-β-glucan present solely for emergency food source in the cell walls? Human being lacks hydrolyzing activity for 1,4- or 1,3-β-linked glucan, but intestinal bacteria probably have such activities. Effect of 1,3,1,4-β-glucan on immunopotentiative action is not well investigated, although it has been well known that 1,3-β-glucan exhibits such action.

Japanese traditional food, Natto, is fermented soybean by Bacillus subtilis var. natto. Since Bacillus subtilis is known for its glucanase activity toward 1,3,1,4-β-glucan, it is highly probable that Bacillus subtilis var. natto also produces such activity. Moreover, Bacillus subtilis produces heat resistant spores that might also facilitate to migrate to human intestinal condition. Therefore, there is a possibility that endo-type 1,3,1,4-β-glucanase activity derived from Bacillus subtilis var. natto is present in intestine and small molecules of degraded 1,3,1,4-β-glucan function in promoting immunopotentiative action.

Brewers has a trouble where the filter is clogged by 1,3,1,4-β-glucan at squeezing process of malt juice. The study had been carried our to select or find a new barley strain that has lower content of 1,3;1,4-β-glucan in the cell walls of barley seed, but in vain. Therefore, plenty of 1,3;1,4-β-glucan is present as an industrial waste.

As for xyloglucans, side chain residues, such as xylose and fucose are α-linked, these linkage are easily hydrolyzed by ubiquitous α-glycosidase. Galactose residue in xyloglu-
Bifidus and lactic acid bacteria are the major bacterial species in the human intestine. However, with the increasing proportion of easily digestible meat, bacteria such as Welch bacilli and E. coli become dominant, promoting intestinal putrefaction. Proteins taken in the body are broken down to amino acids and absorbed in the intestine. Amino acids that are left unabsorbed are anaerobically broken down into foul-smelling degraded products. They are delivered to the liver through the portal vein, contaminating the portal blood. Even in this situation, the liver is required to carry out as much detoxification as possible to deliver clean blood to the body. Detoxified substances are excreted into the duodenum with bile. If intestinal putrefaction is intense, such substances are reduced by enzymes such as β-glucuronidase produced by putrefactive bacteria and are reabsorbed. In other words, detoxified substances, which should be eliminated from the body, are circulated from the intestine to the liver and then back again to the intestine (enterohepatic circulation), accumulating small amounts of carcinogens. When the control of putrefactive bacteria and purification cannot be achieved, the liver needs to keep exerting its detoxification capacity to the fullest, leading to a state that overcomes the liver capacity. This can be one of the major causes of developing digestive system cancer or liver metastasis. There is a conventional belief that the intake of digestive proteins is beneficial in allowing intestinal rest. Intake of large amounts of protein, however, can promote the growth of putrefactive bacteria in the intestine and cause contamination of the portal blood. Therefore, this belief is apparently undesirable from the viewpoint of disease prevention.

I. The significance of intake of indigestible foods.

Dietary fiber is classified into two types, a water-soluble type and a water-insoluble type, which have different behaviors with respect to the human body. While water-soluble dietary fiber does not change the pH in the stomach, the water-insoluble type lowers the pH in the stomach. Water-soluble dietary fiber lowers serum cholesterol and regulates postprandial blood sugar. Meanwhile, water-insoluble dietary fiber increases stool volume and is said to have a greater colon cancer prevention effect than water-soluble dietary fiber. However, considering the results of studies on the colon cancer inhibitory effects of water-soluble dietary fiber, including apple pectin, it seems too early to make a definite conclusion on this matter.

Following oral intake, dietary fiber remains almost completely undigested by digestive enzymes in the body. Therefore, it has been thought that it is nutritionally useless and that excessive intake of fiber imposes an excessive burden on the digestive tract and that it prevents intestinal rest. However, dietary fiber increases the frequency of chewing, which can affect health expectancy, and promotes the secretion of saliva. Since peroxidase, an enzyme contained in saliva, acts as a scavenger of active oxygen, dietary fiber can be directly and indirectly beneficial for health. This invites us to re-define the term “intestinal rest.” Some people understand the term as the presence of no food left in the intestinal tract or as the intake of readily-digestible foods. However, it appears that there is no basis for such interpretations of the term. Some dietary fiber serves as a factor that promotes the proliferation of bifidus, which results in promotion of the production of short-chain fatty...
acids, the energy source for cells in the intestinal tract. This also produces a slightly acidic intestinal environment, preventing the proliferation of putrefactive bacteria and reducing the amount of intestinal putrefactive products. In other words, dietary fiber plays a scavenger role in cleaning the intestinal tract, indirectly preventing leaky gut syndrome and bacterial translocation through the walls of the intestinal tract, and relatively increasing the antioxidative potency of the portal vein blood.

II. Inhibition of carcinogenesis through dietary fiber intake.

In his 1971 report, Burkitt proposed the idea that a lack of dietary fiber was a factor for the development of colon cancer, based on the fact that the incidence of colon cancer and cardiac disease was low amongst the native people of Africa, who consume large amounts of dietary fiber, while the corresponding incidence was high amongst Western people, who consume small amounts of it.

In Japan, the number of patients with colon cancer is also increasing. This is attributable to the Westernization of Japanese dietary habits and grains becoming increasingly refined, with an increase in consumption of animal products and a decrease in the intake of dietary fiber from foods such as vegetables. If digestible food constituents, which are said to be beneficial for intestinal rest, are retained in the intestinal tract, promote the growth of Welch bacilli that putrefy protein, and generate more carcinogens, how should we think about their effect on health?

Further, indigestible dietary fiber adsors carcinogens and substances that promote carcinogenesis, accelerates their elimination from the body, and stimulates bowel peristalsis together with the effect of increasing stool volume, contributing to speeding the passage of foods through the digestive system. In other words, dietary fiber plays the role of a house-keeper for the intestinal tract. In addition, it increases intestinal flora such as bifidus, which inhibits the growth of putrefactive bacteria, and promotes the elimination of feces, accelerating the elimination of carcinogens and toxic substances produced by intestinal flora from the body.

III. Suppression of carcinogenesis and reduction in prostaglandin E2 (PGE2) in the intestinal mucosa and the portal vein by pectin.

Since Burkitt presented the idea that a lack of dietary fiber was one of the factors in the development of colon cancer, many researchers have studied the preventive effects of dietary fiber on colon cancer development. However, these results are not always consistent, depending on the types of dietary fiber. There have been a number of reports on the effectiveness of pectin (soluble fiber). However, most such reports concern citrus pectin (CP) and few of them are about apple pectin (AP). The physiological properties of CP and AP are characterized by their bacteriostatic effects on putrefactive bacteria. AP is superior to CP in terms of bacteriostatic effect. The following data are the results of our study on CP and AP concerning the development of colon cancer.

We examined the inhibitory effect of AP and CP on the development of colon cancer in rats using azoxymethane (AOM). The results of our study found the incidence of cancer to be 100% (19/19) in the control group, 70% (14/20) in the 10% AP group, and 45% (9/20) in the 20% AP group, showing that carcinogenesis was suppressed in proportion to content of AP. In addition, the result of comparison of 20% AP with 20% CP was as follows: The incidence of cancer was 83.3% (15/18) in the control group, 37.5% (9/24) in the 20% AP group, and 55.0% (11/20) in the 20% CP group. It can be clearly seen that the incidence of cancer in the AP group was lower than that in the CP group. The number of tumors developed in individual rats was 0.46 ± 0.18 in the AP group and 1.05 ± 0.29 in the CP group, demonstrating that the former was significantly smaller than the latter (p < 0.001). With regard to enzyme activity in the feces, β-glucosidase was decreased in both the AP group and the CP group and β-glucuronidase was also decreased during the AOM administration period (p < 0.01). In the AP group, a decrease in total bile acid in the feces and a significant decrease in primary bile acid were observed (p < 0.05).

In order to examine the scavenger effect of pectin in the intestinal tract, the level of prostaglandin E2 (PGE2), which increases when inflammation occurs in the wall of the intestinal tract, was measured for the intestinal mucosa and portal vein blood. The content of PGE2 in the distal colon mucosa following oral AP administration was as follows: 422.1 ± 125.6 ng/g in the control group and 166.6 ± 25.8 ng/g in the AP group, which was significantly lower than that in the control group (p < 0.001). Further, the PGE2 level was 324.9 ± 33.7 ng/g in the CP group, which was also significantly lower (p < 0.001). The content of PGE2 in the portal vein blood following oral AP administration was as follows: 0.81 ± 0.17 ng/mL in the control group, 0.54 ± 0.13 ng/mL in the 10% AP group and 0.30 ± 0.08 ng/mL in the 20% AP group, which was further lower (p < 0.05).

As shown in these results related to PGE2, the decrease in PGE2 content in the distal colon mucosa, where cancer often develops, suggested that pectin prevented the decrease in immunity at the local mucosa. This can be interpreted that AP had a scavenger-like effect in the intestinal tract. AP may relatively enhance liver function by purifying the portal blood as suggested by the results. Our study has demonstrated that AP has an active oxygen removal effect. Based on all these results, AP can be viewed as a highly effective cleaner of the intestinal tract.

IV. The significance of apple pectin’s inhibition of liver metastasis.

The levels of PGE2 in the large-intestinal mucosa and the portal blood after oral AP administration were significantly lower than those in the basic diet group. Therefore, we investigated how AP affected the liver immunologically in the wall of the intestinal tract, was measured for the intestinal mucosa and portal vein blood. The content of PGE2 in the distal colon mucosa following oral AP administration was as follows: 422.1 ± 125.6 ng/g in the control group and 166.6 ± 25.8 ng/g in the AP group, which was significantly lower than that in the control group (p < 0.001). Further, the PGE2 level was 324.9 ± 33.7 ng/g in the CP group, which was also significantly lower (p < 0.001). The content of PGE2 in the portal vein blood following oral AP administration was as follows: 0.81 ± 0.17 ng/mL in the control group, 0.54 ± 0.13 ng/mL in the 10% AP group and 0.30 ± 0.08 ng/mL in the 20% AP group, which was further lower (p < 0.05).

As shown in these results related to PGE2, the decrease in PGE2 content in the distal colon mucosa, where cancer often develops, suggested that pectin prevented the decrease in immunity at the local mucosa. This can be interpreted that AP had a scavenger-like effect in the intestinal tract. AP may relatively enhance liver function by purifying the portal blood as suggested by the results. Our study has demonstrated that AP has an active oxygen removal effect. Based on all these results, AP can be viewed as a highly effective cleaner of the intestinal tract.
Studies of foods that provide adequate fiber. This phenomenon can be explained as follows. AP inhibited the production of PGE2 in the intestinal tract and decreased the generation of active oxygen. The portal vein blood was purified by this highly effective scavenger-like effect of pectin, inhibiting cancer metastasis by activating immunity in the liver. This demonstrated that the intake of indigestible fiber, in contrast to the conventional idea of intestinal rest, cleaned the intestinal mucosa and the portal vein blood and played an important role in preventing cancer metastasis in the liver. More specifically, the currently recommended post-surgical diet is focused on foods containing low amounts of fiber and high amounts of protein which can be easily absorbed. It typically starts with a liquid diet, the patient is then served a thin gruel, then normal gruel, thin porridge, and then normal porridge before being served normal diet.

Nutritional management with a focus on the intestinal environment can enhance the function of the intestinal tract and improve intestinal flora, which eventually increases cellular energy production and the function of all internal organs. This enhances the patient’s ability to defend against infections, and, ultimately, will decrease the incidence of complications and mortality. Finally, I would like to emphasize that the intake of dietary fiber is effective not only in the prevention of cancer development and metastasis but also in many other ways.

CONCLUSION

In the field of nutritional science, emphasis has traditionally been placed on absorbable food components while indigestible or poorly absorbable food components have been viewed negatively. Such science is apparently questionable. From now on, it will be necessary to modify these conventional views of nutrition and to work seriously on the potential effects of dietary fiber on the prevention of cancer development or post-surgical liver metastasis. Modern medicine should also be aware of how important a role the foods we consume on a daily basis play in cancer treatment.

**Dietary Fiber: Current Policy and Future Directions**

Barbara Schneeman\(^1,*\)

\(^1\)Center for Food Safety and Applied Nutrition, the Food and Drug Administration (5100 Paint Branch Parkway, College Park, MD 20740, USA)

The Dietary Guidelines for Americans continues to emphasize the importance of consuming fiber-containing foods, including whole grains, fruits, vegetables, beans and legumes. Data from NHANES suggests that many Americans do not consume the recommended servings of these foods nor meet the Adequate Intake levels established by IOM for dietary fiber intake. Since the 1990s the Dietary Guidelines for Americans have emphasized the importance of consuming foods that provide adequate fiber.

Since 1994, nutrition information has been required on most packaged foods in the United States. Dietary fiber is included in the list of mandatory nutrients for food labels and is listed as a sub-category under Total Carbohydrates in Nutrition Facts. In developing its regulations for the declaration of dietary fiber, FDA referenced the definition of dietary fiber in the 1987 report published by the Life Sciences Research Office (LSRO). For compliance purposes FDA refers to the 15th edition of AOAC Official Methods. This method includes nonstarch polysaccharides, lignin, and some resistant starch. FDA also issued regulations for making nutrient content claims and health claims in food labeling. Nutrient content claims are based on the nutrient profile of a product and health claims describe a relationship between a food substance and a disease or health-related condition. Both nutrient content claims and certain types of health claims are recognized for dietary fiber or dietary fiber containing foods.

In 2005 the National Academy of Sciences-Institute of Medicine (IOM) published the Dietary Reference Report (DRI) for Macronutrients, which included recommendations for dietary fiber. IOM recognized two terms for the total fiber content of a food, dietary fiber as nondigestible carbohydrates and lignin that are intrinsic and intact in plants and functional fiber as isolated, nondigestible carbohydrates that have beneficial physiological effects in humans. In 2007 FDA published in the Federal Register a notice of advanced rule making that requested information about updating the reference values used for nutrition labeling and specifically requested input on how to utilize recommendations in the IOM DRI reports to update nutrition labeling. FDA is in the process of reviewing this information and developing a proposed rule to update nutrition labeling.

**Hydrolized Hemicellulose from Purple Rice Prolongs Survival in Cancer Patients**

Hiroshi Tsunekawa\(^1,*\)

\(^1\)Tsunekawa Clinic (1–22–13 Taiko, Nakamura-ku, Nagoya 453–0801, Japan)

**INTRODUCTION**

We have been applying complementary and alternative medicine (CAM) for the treatment of patients with advanced progressive cancer to enable recovery and restoration of the immune system to promote self healing. Almost all of the studied patients were undergoing CAM treatment to increase their ability to recover their quality of life during and after conventional cancer treatments. Since many food components have been reported as effective biological response modifiers (BRM) for enhancing the immune system, we recommend an intake of functional food to enhance the effects of CAM treatment. In particular, many indigestive polysaccharides, such as β-glucan which originates from mushroom and yeast and fucoidan which originates from seaweed, have been reported. A number of patients at our clinic are continuously taking a hydrolized hemicellulose called oryzalose—an indigestive polysaccha-
ride—which originates from purple rice. A total of 36 patients in the advanced progressive cancer stages III and IV who were treated with CAM and were taking oryzalose over the long term showed remarkably prolonged life.

**MATERIAL AND METHODS**

**CAM treatments.** An oriental CAM treatment containing the following ingredients for increasing recovery ability and enhancing the immune system was used: Hochuekkito, Juzentaihodo, Ninjinyocito among others and high-potency vitamin C injection (20–50 g per day, 1–2 times per week or 2–3 times per month). Patients. Cancer patients in the advanced progressive III and IV clinical stages who had been treated with conventional therapy were treated with our CAM therapy from August 2004 to May 2011. Patients also received oryzalose as a functional food for restoration of the immune system. Site of primary onset, clinical stage, sex, and average age are shown in Table 1.

**Oryzalose.** Oryzalose is a functional food developed and processed in Japan (Origin Biochemical Inc., Tokyo, Japan) and is derived from hemicellulose in the seed coats of purple rice. The major active components are oligosaccharides and polysaccharides contains onto cyawn a kind of ply phenol, which are hydrolysates of hemicellulose, and comprise mainly pentose sugars such as arabinoxylan, arabino galactan, arabinoxylagalactan, and arabinan. These heteroglycan complexes constitute oryzalose. The molecule weight is estimated to be around 1,000–10,000 Da. Functions reported for oryzalose include restoration of the immune system, and antioxidant and anti-aging effects.

**Evaluation of Patient Nutritional Condition.** Many cases of malnutrition are observed in cancer patients in the advanced progressive stage, and this leads to a lowered quality of life (QOL) because of the functional disturbance in metabolism. Two indicators, serum albumin and choline esterase, were adopted for the evaluation of nutritional conditions. These indicators were monitored monthly and indicated annual variations from the beginning of CAM treatment in survivors and bi-annual variation until death in non-survivors. Grade was scored on a 4-point scale (4=change within normal range, 3=change from normal to abnormal, 2=change from normal to abnormal, 1=changes within abnormal range). Better conditions achieved a higher score.

**Evaluation of immune potential.** It is important to maintain immune potential in advanced progressive cancer patients to improve QOL and vitality. Two indicators of variation, white blood cell number (WBC) and natural killer cell activity (NK), were adopted for the evaluation of immune potential. WBC count and NK activity in all patients were monitored monthly. The $^{51}$Cr-released assay was used to determine NK cell activity. These indicators showed annual variations from the beginning of CAM treatment in survivors and bi-annual variation until death in non-survivors. Evaluation of NK activity was based on the activity grade of NK during the test period and on the improvement after treatment.

**Monitoring of Tumor Associated Antigens.** Each patient was monitored monthly for two or three kinds of Tumor Associated Antigens (TAA) during CAM treatment. The main TAA were ovarian (CA125), prostate (PSA), breast

### Table 1. Patient data.

<table>
<thead>
<tr>
<th>Primary onset location</th>
<th>Stage III</th>
<th>Stage IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Breast</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Large intestine</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Lung</td>
<td>–</td>
<td>4</td>
</tr>
<tr>
<td>Pancreas</td>
<td>–</td>
<td>3</td>
</tr>
<tr>
<td>Esophagus</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Other</td>
<td>4</td>
<td>3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sex</th>
<th>M6/F8</th>
<th>M11/F12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (average years)</td>
<td>M61.5/F51.2</td>
<td>M63.7/F66.6</td>
</tr>
</tbody>
</table>

### Table 2. Details of stage IV patients.

<table>
<thead>
<tr>
<th>Patient initials</th>
<th>Primary site</th>
<th>Metastasis</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Survival time (months)</th>
<th>Status as of May 2011</th>
</tr>
</thead>
<tbody>
<tr>
<td>OY</td>
<td>Breast</td>
<td>lung, liver</td>
<td>F</td>
<td>57</td>
<td>230</td>
<td>Survivor</td>
</tr>
<tr>
<td>IK</td>
<td>Cutaneous</td>
<td>recurrence</td>
<td>M</td>
<td>65</td>
<td>154</td>
<td>Survivor</td>
</tr>
<tr>
<td>KM</td>
<td>Esophageal</td>
<td>jugular lymph node</td>
<td>M</td>
<td>72</td>
<td>135</td>
<td>Survivor</td>
</tr>
<tr>
<td>YY</td>
<td>Lung</td>
<td>brain</td>
<td>M</td>
<td>70</td>
<td>132</td>
<td>Survivor</td>
</tr>
<tr>
<td>NN</td>
<td>Breast</td>
<td>lung, bone</td>
<td>F</td>
<td>66</td>
<td>114</td>
<td>Survivor</td>
</tr>
<tr>
<td>KK</td>
<td>Breast</td>
<td>lung, liver</td>
<td>F</td>
<td>68</td>
<td>109</td>
<td>Survivor</td>
</tr>
<tr>
<td>SA</td>
<td>Colorectal</td>
<td>lung, liver</td>
<td>F</td>
<td>59</td>
<td>89</td>
<td>Survivor</td>
</tr>
<tr>
<td>UM</td>
<td>Lung</td>
<td>jugular lymph node</td>
<td>M</td>
<td>56</td>
<td>85</td>
<td>Survivor</td>
</tr>
<tr>
<td>KE</td>
<td>Lung</td>
<td>mediastinal lymph node</td>
<td>F</td>
<td>68</td>
<td>81</td>
<td>Survivor</td>
</tr>
<tr>
<td>MA</td>
<td>Breast</td>
<td>bone</td>
<td>M</td>
<td>74</td>
<td>77</td>
<td>Survivor</td>
</tr>
<tr>
<td>HR</td>
<td>Colorectal</td>
<td>lung, peritoneum lymph node</td>
<td>F</td>
<td>71</td>
<td>71</td>
<td>Survivor</td>
</tr>
<tr>
<td>AH</td>
<td>Breast</td>
<td>liver</td>
<td>M</td>
<td>68</td>
<td>70</td>
<td>Survivor</td>
</tr>
<tr>
<td>YH</td>
<td>Ovarian</td>
<td>peritoneum lymph node</td>
<td>F</td>
<td>53</td>
<td>67</td>
<td>Survivor</td>
</tr>
<tr>
<td>KT</td>
<td>Testicular</td>
<td>peritoneum lymph node</td>
<td>M</td>
<td>50</td>
<td>65</td>
<td>Survivor</td>
</tr>
<tr>
<td>MU</td>
<td>Breast</td>
<td>Breast</td>
<td>F</td>
<td>51</td>
<td>62</td>
<td>Survivor</td>
</tr>
<tr>
<td>SY</td>
<td>Pancreatic</td>
<td>lung, liver</td>
<td>M</td>
<td>68</td>
<td>33</td>
<td>Non-survivor</td>
</tr>
<tr>
<td>NM</td>
<td>Colorectal</td>
<td>liver</td>
<td>M</td>
<td>64</td>
<td>28</td>
<td>Non-survivor</td>
</tr>
<tr>
<td>YN</td>
<td>Gastric</td>
<td>lung</td>
<td>M</td>
<td>78</td>
<td>25</td>
<td>Non-survivor</td>
</tr>
<tr>
<td>FN</td>
<td>Gastric</td>
<td>peritoneum lymph node</td>
<td>F</td>
<td>45</td>
<td>17</td>
<td>Non-survivor</td>
</tr>
<tr>
<td>OH</td>
<td>Gastric</td>
<td>peritoneum lymph node</td>
<td>M</td>
<td>53</td>
<td>16</td>
<td>Non-survivor</td>
</tr>
<tr>
<td>SJ</td>
<td>Pancreas</td>
<td>peritoneum lymph node</td>
<td>M</td>
<td>63</td>
<td>15</td>
<td>Non-survivor</td>
</tr>
<tr>
<td>FM</td>
<td>Pancreas</td>
<td>liver</td>
<td>F</td>
<td>71</td>
<td>8</td>
<td>Non-survivor</td>
</tr>
<tr>
<td>KK</td>
<td>Gastric</td>
<td>liver</td>
<td>M</td>
<td>59</td>
<td>7</td>
<td>Non-survivor</td>
</tr>
</tbody>
</table>

Male, n=11; Female, n=11. Average age, 63.3 ± 8.7 years.
Variation in TAA was monitored annually from the beginning of CAM treatment in survivors and bi-annually until death in non-survivors. Evaluative grade was scored on a 4-point scale (4=change within normal range, 3=change from abnormal to normal, 2=change from normal to abnormal, 1=changes within abnormal range).

RESULTS AND DISCUSSION

In stage III, the maximum survival period was 114 months and the survival rate was 78.6% for the 8 survivors and 3 non-survivors. In stage IV, the maximum survival period was 230 months for the 14 survivors and 114 months for the 8 non-survivors, and the minimum survival period was 7 months.

The average age of patients in stage IV was 63.3 ± 8.7 and the average survival period was 73.7 ± 56.3 months with a five-year survival rate of 63.6%. The survival curve is shown in Fig. 1. The nutritional condition of all patients in stage III, both survivors and non-survivors, was the same as that of healthy persons. The survivors in IV stage had mainly a normal nutritional condition, the same as that of the healthy persons.

When immune response was evaluated, the difference in NK activity between survivors and non-survivors was observed clearly in all patients in the same clinical stage. In the case of stage IV, indicators of immune potential and total WBC in survivors were mainly normal, although NK cell activity generally indicated low level, and 6 of 14 patients improved during the CAM treatment period. For non-survivors, immune potential apparently decreased, and total WBC in 7 of 8 patients indicated abnormal immune potential, while NK activity also indicated abnormal immune potential, was observed in 5 patients. This result demonstrates a significant difference between stages III and IV regarding nutritional condition and immune function. Irreversible metabolic dysfunction was not observed in subjects during stage III but was often observed in non-survivors during stage IV.

TAA evaluation showed that the majority of all patients in stage III and survivors in stage IV shifted around the normal range. On the other hand, the majority of non-survivors in stage IV shifted beyond the limit. The significant difference in TAA score indicates the condition grade.

The advanced progressive cancer patients who were treated with our CAM combination therapy, which included administration of oryzalose, showed an extended period of survival. Worthy of special mention is the five-year survival rate (63.6%) of the patients in stage IV.

The general five-year survival rate of cancer patients in stage IV who were diagnosed from 1997 to 1999 in Japan was reported in 2006. According to this report, the survival rate is 5.6% for patients with stomach cancer, 12.0% for colon cancer, 19.9% for liver cancer, 28.4% for breast cancer, and 38.0% for prostate cancer, with an average survival rate of 20.8% for major cancer. In relation to this report, the extensive effect of oryzalose-based CAM treatment on survival period is obvious.

CONCLUSION

The most important result of the present study is the high survival rate observed in patients, even in those in stage IV, with no great difference between stages being observed. This result suggests that CAM treatment and the immune restorative effect of oryzalose as a BRM are effective for improving the healing ability of advanced progressive cancer patients. In future studies, we intend to continue observation of these patients and to increase the number of evaluated clinical cases.

*E-mail: d_tsune@nifty.com

Research on Elucidation of Functions of Agricultural Products and Foods in NARO & NFRI

Kiyoshi Hayashi 1,*

1National Agriculture and Food Research Organization (NARO), NARO Food Research Institute (NFRI), (2–12 Kannondai, Tsukuba 305–8642, Japan)

1. National Agriculture and Food Research Organization (NARO) & NARO Food Research Institute (NFRI).

NARO, an incorporated administrative agency under the Ministry of Agriculture, Forestry, and Fisheries, plays a key role in the field of agricultural research in Japan. NARO has
total 22 research projects and approximately 1,600 research staff across 14 research institutes throughout Japan, some of which contribute to regional innovations on in the field of agricultural research. NARO Food Research Institute (NFRI) is one of them and specialized on food research.

The mission of NARO is closely related to people’s lives, including disaster management, maintenance technology for production infrastructures in rural areas, crop and livestock breeding and raising, food processing technologies, food and animal safety, and biofuel production and its utilization technologies covering the whole research area related to agriculture and food science in Japan.

Starting from April 2011, NARO has launched a new third five-year mid-term plan. In this plan, research and development for the following purposes are focused: (1) securing a stable food supply, (2) addressing global-scale issues such as climate change, (3) creating demand for new markets and future industries, and (4) utilizing local agricultural resources.

2. Research and development to create demand for new markets and future industries.

Of these 4 focused purposes, “(3) creating demand for new food products” is consisted with 3 research projects as follow:

A. Elucidation of functions of agricultural products and foods and development of a provision system for obtaining reliable information
B. Development of high quality agricultural products
C. Development of an integrated process of distribution and processing for value-added agricultural products and foods

3. Elucidation of functions of agricultural products and foods and development of a provision system for obtaining reliable information.

In our research for creating demand for new foods, we study the nutritional function of food in human health and develop foods and foodstuffs with enhanced functionality. As the “medicine and one’s daily diet are equally important for a healthy body” indicates, many specific substances in food might have the function of risk reduction of lifestyle-related diseases, anti-allergic activity and anti-aging effects. By promoting cooperative works with medical science, we hope to enhance scientific evidence in the function of food and strengthen them. Further, we develop technologies to evaluate the sensory properties, such as texture and taste of agricultural products and foods. This program is consisted with the following 4 sub-themes.

3-1. Development of standardized methods for analysis of functional components and for evaluation of the functionalities of agricultural products and foods.

For building databases of functional ingredients contained in local agricultural products and foods, this program aims to validate and standardize analytical technology for evaluating the functional ingredients and factors such as the total anti-oxidant capacity. Moreover, several previously developed evaluation technologies for functional factors such as nutrigenomics will also be utilized to evaluate function and safety of the ingredients.

Hydrophilic oxygen radical absorbance capacity (H-ORAC) is one of the most recognized indices for evaluating anti-oxidant capacities of hydrophilic components. However, the original H-ORAC method possesses relatively low reproducibility. Therefore, we modified the method and evaluated the precision of the improved method by an inter-laboratory test using anti-oxidant solutions and food extracts. These results indicated sufficient precision of the H-ORAC method.

3-2. Elucidation and effective utilization of metabolic regulatory functions of foods.

This program aims to elucidate the metabolic regulatory functions that prevent metabolic syndromes and other lifestyle-related diseases and to evaluate safety of agricultural products from the National Agriculture and Food Research Organization’s (NARO’s) genetic resources using methods such as gene expression analysis and animal disease models. For this purpose, new evaluation methods to assess metabolic regulatory functions will be developed. The active components, which show metabolic regulatory functions, and their content in agricultural products will be clarified. To elucidate metabolic regulatory functions of fruit components, an intervention trial and a study using animal models of diseases will be conducted. In addition, effective combinations of functional food components will be clarified. Foods rich in components with metabolic regulatory functions will be developed.

Deoxyribonucleic acid (DNA) microarray carrying

Topic 2. Development and utilization of a deoxyribonucleic acid (DNA) microarray to assess the allergic or anti-allergic property of food components.
approximately 200 genes related to immunity and inflammation has been developed and used to find the effect of bitter gourd extract on allergic or inflammatory cells.

3-3. Elucidation and effective utilization of the functionalities of foods against homeostatic disturbances.

We will develop new technologies to evaluate food functionality of agricultural products with regard to health-promoting functions. Our targets include anti-oxidative activity, which is thought to be involved in preventing various diseases, anti-allergic activity such as immune regulation, and anti-aging effects. We will accumulate evidence regarding safety and effectiveness of bio-active ingredients using various experimental techniques such as cultured cells, animal disease models, and human clinical trials. Moreover, we will propose effective methods of food intake that fully utilize its functionality and will develop effective utilization technology for food functionality. Based on these outcomes, we will develop foods or food materials that could increase healthy life expectancy of the super-aging society in future and also reduce medical costs because of increase in immune-related disorders.

We elucidated the inhibitory mechanism of EGCG3ζMe on mast cell activation. In addition, we analyzed the characteristics of ‘Benifuuki’ tea leaves and evaluated the anti-allergic effect of ‘Benifuuki’ green tea in humans.

3-4. Evaluation technologies for flavor, texture, and other sensory properties of foods.

In this research project on quality and sensory properties, objective evaluation technologies for flavor, texture, and other sensory properties of agricultural products and foods will be developed and improved. Practical labeling and information and communication technologies will also be proposed to help increase consumer understanding about the quality of agricultural products and foods.

Texture terms of 445 were collected through questionnaires to food scientists. Questionnaires were also administered to Japanese consumers, and 135 terms were considered as consumer vocabulary.

REFERENCE


*E-mail: hayashi@toyo.jp

**********

Regulation of T Cell Immune Response by Oral Administration of β-Glucan

Noriko M. Tsuji 1,*
1Biomedical Research Institute, National Institute of Advanced Industrial Science and Technology (AIST)
(1–1–1 Tsukuba Central 6, Higashi, Tsukuba 305–8566, Japan)

Dendritic cells as a key player of protective immunity.

Immune system is essential to protect our organs and life from infection and/or cancer. Accordingly, phagocytotic cells such as neutrophils, macrophages, and dendritic cells present antigens to T cells and induce antigen-specific cytotoxic T cells and antibodies to combat pathogens. In similar manner, transformed cells are recognized as abnormal/non-self cells and eliminated by immune system thus preventing malignancy. Dendritic cells are believed to play the central role to present antigen from both pathogens and cancer cells. Concurrent activation of other innate immune cells such as NK cells and NKT cells is also important to induce cytokine secretion, thereby facilitating the induction of protective immunity.

Innate immune cells not only phagocyte pathogens and produce antimicrobial components, they also present antigens to T cells and provide co-stimulatory signal as well as secrete cytokines to induce acquired immunity. In short, the interaction between innate and acquired immune system is inevitable to protect body from infection and/or cancer.

Oral tolerance.

The gut acts to tolerate harmless antigens yet remains able to eliminate pathogens. Food antigens are presented to the mucosal immune system in a tolerogenic manner. Exogenous antigens are processed by the gut associated lymphoid tissue (GALT) system and utilized for an active suppression mechanism to render the host immunologically unrespon-
sive to them. Thus the GALT employs mechanisms to tolerate, or exist in symbiosis with, a large innocuous community of gut microbes. Even if non-self antigens, food components such as cow’s milk or hen’s egg proteins are recognized as pseudo-self, i.e. as safe as self-components via induction of regulatory T cells. Dendritic cells, which present antigens to T cells for induction of regulatory T cells, are essential for the establishment of oral tolerance and called tolerogenic dendritic cells. In the absence of such tolerogenic mechanisms, foreign antigens in diet should evoke effector T cell immune response, resulting in unwanted inflammation (food allergy).

Amplification of immune response or stabilization of anti-inflammatory response by oral administration of polysaccharide.

*Kjellmaniella crassifolia* Miyabe (gagome) is a brown algae. Oral gagome administration (oral gagome) resulted in significant upregulation of IL-10 and IFNγ production by Peyer’s patch cells. To assess the adjuvant activity of oral gagome, treated mice were subcutaneously injected with ovalbumin (OVA). The production of cytokines from antigen-specific T cells in draining lymph nodes (dLN) and their proliferative response were significantly increased as compared with the control group. These enhancements were associated with increased CD44(hi)CD62L(−) activated/memory T cells in dLN as well as upregulation of Ag-specific IgA level in luminal contents. No upregulation of cytokine production by dLN T cells was observed in dectin-1-deficient mice, suggesting that the effect of gagome on cytokine production is largely dependent on the dectin-1 pathway despite its composite constituents. Our findings indicate that gagome is an effective immunomodulator and a potent adjuvant for both the intestinal and the systemic immune response.

Not only protective immunity, anti-inflammatory mechanisms are fortified and stabilized by innate immune signals. We found that oral administration of β-glucan derived from Candida albicans cell wall stimulated DCs from Peyer’s patches (PPs) and stabilized anti-inflammatory response in the β-glucan-fed mice (unpublished data). Utilizing such knowledge to enhance Ag-specific immune responses and/or prevent inflammatory diseases through diet is a new paradigm to targeting immune dysfunction.

This work was supported by the Programme for Promotion of Basic and Applied Researches for Innovations in Bio-oriented Industry.

REFERENCES


3) H. Yan, N. Ohno and N.M. Tsuji: The role of C-type lectin receptors in immune homeostasis. *Int. Immunopharm.*, (in press).

*E-mail: nm-tsuji@aist.go.jp