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Meeting Report

Molecular Mechanisms for Inflammation-promoted Pathogenesis of Cancer—The Sixteenth International Symposium of the Sapporo Cancer Seminar[1]

Inflammation is a necessary process by which the body rejects microbial invaders. Unfortunately, in some circumstances, inflammation persists without attaining its foremost objective. Inflammatory cells chronically inundate an organ system and, in the process, damage normal host cells. Virtually all clinicians recognize the link between chronic inflammation and cancer. Whether the underlying process be colitis, chronic skin ulceration, hepatitis, pancreatitis, cystitis, or gastritis, the long-term outcome is frequently malignancy. Although this association is well accepted, it is poorly understood. The purpose of the Sixteenth Sapporo Cancer Seminar, as set forth by Dr. H. Kobayashi (The Sapporo Cancer Seminar Foundation, Sapporo, Japan) and Dr. Hosokawa (Hokkaido University School of Medicine, Sapporo, Japan), was to dissect what is currently known about the pathogenesis of inflammation-related cancer and to set a course for future collaborative research in this multidisciplinary field.

A historical context for this year's topic was introduced in the opening keynote address by Dr. T. Sugimura (National Cancer Center, Tokyo, Japan). The inflammation-malignancy link dates back to the days of Virchow. Early experiments, many of which were performed in Japan, focused on application of irritative substances to the skin of animals, imitating the observation linking coal tar to scrotal cancer in chimney sweeps. The connection between inflammation and malignancy was ultimately brought to the forefront by Peyton Rous and others in the 20th century. It wasn't until very recently, however, that the genetic consequences of inflammation began to be recognized. Dr. Sugimura advanced the multistep carcinogenesis view that exogenous carcinogens magnify the opportunity for chronic inflammation to progress to cancer.

Dr. J. Parsonnet (Stanford University, Stanford, CA) focused on the litany of infectious agents involved in carcinogenesis. These include papillomaviruses, EBV, hepatitis viruses B and C, human T-cell lymphotrophic virus I, *Helicobacter pylori*, *Mycobacterium ulcerans*, *Salmonella typhi*, *Schistosoma hematobium* and *S. mansoni*, *Opisthorchis viverrini*, *Clonorchis sinensis*, and microbes that cause chronic osteomyelitis or cystitis. She accentuated the importance of these infections in carcinogenesis, particularly in developing countries. At present, she estimated that between one-fourth and one-fifth of cancers worldwide were attributable to infection. Next to smoking, she considered infection the most important modifiable factor worldwide in determining cancer incidence. Of interest, Dr. Parsonnet noted that germ-free animals live up to twice as long as conventional animals. She suggested that the day-to-day colonization of our bodies with normal flora comes at a considerable cost.

After these introductory talks, five research themes were outlined for the program: (*a*) specific microbial agents linked to malignancy were described, and outlines of their putative carcinogenic mechanisms were put forth; (*b*) experimental models for inflammation and cancer discussed; (*c*) the role of cytokines in inflammatory carcinogenesis was presented; (*d*) the role of reactive oxygen and nitrogen species in inducing genetic and epigenetic changes was described; and (*e*) novel prevention strategies for inflammation-related malignancy were proffered.

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Microbial Infection and Cancer

The session on microbial infection initially focused on gastric cancer, a leading cause of cancer death in the host country of Japan. Two infectious pathogens were strongly linked to this malignancy: EBV and *H. pylori* infection. Dr. T. Osato (Health Science University of Hokkaido, Hokkaido, Japan) opened the session with his fascinating work on EBV and gastric carcinoma. His group has found that 7% of gastric tumors contain EBV genomic sequences. The sequences are particularly prominent in an unusual form of gastric carcinoma, undifferentiated lymphoepithelioma-like carcinomas, but can also be found in poorly, moderately, and well-differentiated forms of adenocarcinoma. The EBV-containing carcinoma cells seemed to be clonal, with single-fused terminal fragments in those cells that express EBV-determined nuclear antigen I (other Epstein Barr nuclear antigens were not expressed). Interestingly, these cells were found to not express the cytotoxic T-cell target antigens, suggesting that EBV-infected malignant clones are able to survive, in part, by evading normal immune detection and destruction. Dr. Tokunaga (Kagoshima City Hospital, Kagoshima, Japan) further expounded on the role of EBV in gastric carcinoma. Using EBV-encoded small RNA *in situ* hybridization, he identified monoclonal episomes of EBV in a significant proportion of tumors. Dr. Tokunaga reiterated the important role for this virus in lymphoepithelioma-like tumors but also highlighted the lace-like pattern observed in early gastric carcinomas associated with EBV. Dr. Tokunaga speculated that EBV may also play a role in certain gastric cancer precursor lesions. In particular, he noted the occasional presence of EBV in dysplastic, metaplastic, and atrophic tissue.

Dr. J. Parsonnet presented a different view of gastric carcinogenesis, focusing on bacterial rather than viral processes. She briefly reviewed the evidence that led the IARC to declare *H. pylori* a Group I carcinogen, a definite cause of cancer in humans. Although she did not discount that *H. pylori* may be sufficient to cause cancer in some people, Dr. Parsonnet reiterated Dr. Sugimura's view that multiple factors are probably required for carcinogenesis in the majority of cases. This is corroborated by the rarity of cancer even among *H. pylori*-infected persons. Four factors were thought to contribute to carcinogenesis in infected persons: (*a*) the duration of infection; (*b*) the presence of other carcinogenic environmental exposures (such as EBV); (*c*) the virulence of the *H. pylori* strain; and (*d*) host genetics. Mechanisms of carcinogenesis imputed to *H. pylori* are its proinflammatory nature, inducing cytokine release and free radical formation, and its role in increasing cell division by causing both apoptotic and necrotic cell death. Because *H. pylori* is curable with antibiotics, studies can address cancer prevention using *H. pylori* therapy.

The role of *H. pylori* in gastric cancer in Japan was reviewed by Dr. M. Asaka (Hokkaido University School of Medicine). His group found that *H. pylori* antibodies were statistically associated with both common histological types of gastric adenocarcinoma, the diffuse and

intestinal types. *H. pylori* antibodies were particularly prevalent in persons with early gastric carcinomas. In this group, 93% of patients had evidence of *H. pylori* infection; this was five times higher than the prevalence observed in normal controls. Advanced gastric cancer patients were found to have only modestly higher prevalence of *H. pylori* antibodies than controls, suggesting that as disease advances, both infection and antibodies are lost.

Dr. S. Satarug (University of Arizona, Tucson, AZ) and Dr. W. Anwar (Ain Shams University, Cairo, Egypt) presented the parasites' role in the chronic infection to malignancy cascade. Dr. Satarug presented data on *O. viverrini* in Thailand. She first related the development of cholangiocarcinoma to the magnitude of infection as determined by eggs excreted in the feces. She then discussed ongoing efforts to look at nitrosation by endogenous nitric oxide as a putative mechanism of carcinogenesis.

Dr. Anwar described the frightening magnitude of schistosomiasis in Egypt and worldwide. In Egypt, bladder carcinomas constitute 50% of all malignancies. That *S. hematobium* and bladder cancer are linked was demonstrated by the loss of microsatellite instability in exfoliated bladder epithelial cells in persons treated for schistosomiasis with praziquantal. As with other chronic infections, she emphasized the important role of environmental cofactors in determining infection outcome. In particular, she noted the high levels of smoking, bacterial cystitis, and exposure to environmental pollutants in persons with schistosomiasis-related malignancy. She also discussed the role of genetic cofactors, presenting new data on genetic polymorphisms in glutathione-*S*-transferases as markers for increased disease risk. Her group found that persons with the null phenotype of both GSTM1 and GSTT1 have markedly higher rates of Schistosome-related malignancy. No such relationship was observed between cancer and cytochrome P450 polymorphisms.

Experimental Models for Cancer Caused by Inflammation

In experimental models of carcinogenesis, the principal focus was hepatitis and hepatocellular carcinoma. Dr. M. K. Shigenaga presented for T. M. Hagen of the same institute (University of California, Berkeley, CA), a fascinating transgenic mouse model of hepatitis B virus infection developed by Francis Chisari of the Scripps Research Institute. This transgenic mouse expresses the large envelope protein of hepatitis B virus on hepatocytes, resulting in a necroinflammatory, ground-glass hepatitis that mimics human disease. Moreover, this mouse progresses to hepatic adenoma and hepatocellular carcinoma. Histopathologically, the livers of these mice showed high levels of superoxide formation in Kupffer cells that corresponded with formation of oxidative DNA adducts (8-hydroxy-2′-deoxyguanosine) in neighboring parenchymal cells. This was particularly evident in mice with nodular hyperplasia, adenomas, or hepatocellular carcinoma. In contrast, levels of tissue antioxidants (reduced glutathione, catalase) were depleted. Inflamed areas also manifested high rates of cell proliferation. In preliminary treatment experiments, *N*-acetylcysteine supplementation spared the drop in glutathione and also reduced oxidative DNA damage compared to those in unsupplemented transgenic mice. These experiments supported a role for oxidative damage in the causal pathway of inflammation-related carcinogenesis.

Two groups, that of Dr. M. Mori (Sapporo Medical University, Sapporo, Japan) and that of Dr. T. Moriuchi (Hokkaido University School of Medicine), expounded further on liver injury in an animal model, presenting data on LEC[2] mutant rats, a rat strain that spontaneously develops hepatitis and hepatocellular carcinoma. Analogous to Wilson's disease in humans, liver damage in LEC rats is marked by accumulations of copper due to a mutation in the gene for a copper-transporting ATPase gene (*Atp7b*). Paradoxically, Dr. Mori's group has found that cells containing substantial quantities of copper have low proliferation rates and do not progress to malignancy. In contrast, initiated cells with little copper accumulation are in a hyperproliferative state and develop adenomas and cancers. When compared with LEC rats fed a copper-deficient diet, LEC rats fed a normal diet showed decreased responsiveness to proliferative stimuli and evidenced higher expression of p53 and p21. Mori surmised from these experiments that cells that accumulate copper induce hepatitis. The inflammation then promotes tumor development in adjacent, spontaneously initiated hepatocytes. Indeed, previous work from Dr. Mori's lab suggests that cancer is largely independent of the presence of copper in the hepatocytes.

LEC rats were further studied in Dr. Moriuchi's laboratory. He showed that these rats have deficiencies in hepatic glutathione peroxidase but not in other tissue peroxidases. He speculated that this deficiency promotes oxidative damage and increased cancer risk. Although the gene for glutathione peroxidase was present in the rats, transcription was diminished. Interestingly, the promoter for glutathione peroxidase seemed to be turned on by p53 protein. Because p53 is not mutated in LEC mice, however, Dr. Moriuchi concluded that some as yet unidentified factor diminishes up-regulation of glutathione peroxidase by p53 in LEC animals. Dr. Moriuchi further reported that glutathione peroxidase deficiency was not apparent in LEC Atp7b congenic rats, even though these rats continue to accumulate copper in the liver. These findings, like those of Dr. Mori, support a carcinogenic mechanism in LEC rats distinct from the *Atp7b* gene.

The final animal model presented was a rat bladder cancer model from the laboratory of Dr. R. Oyasu (Northwestern University Medical School, Chicago, IL). Dr. Oyasu first reviewed the increasingly strong epidemiological data linking chronic and recurrent urinary tract infection to bladder carcinoma. He then presented a mouse model in which bladders were heterotopically transplanted. When exposed to killed *Escherichia coli* or its lipopolysaccharide with MNU, 100% of transplanted bladders developed tumors; this compared with only 10–16% of the bladders exposed to *E. coli* or MNU alone. The bladders treated with *E. coli* and MNU instillation had greater polymorphonuclear leukocyte infiltration as well as higher levels of hydrogen peroxide, TNF, IL-1, and IL-6 than did those treated with MNU only. To see if the tumorigenic response to killed *E. coli* was due to free radical damage or cytokine stimulation, Dr. Oyasu devised a tissue culture model using MYP3 cells, an anchorage-dependent, nonmalignant rat bladder epithelial cell line. MYP3 cells grow in soft agar only when transformed. Dr. Oyasu found that hydrogen peroxide, with and without MNU, was capable of transforming MYP3 cells. Subsequent growth of cells was greatly enhanced by IL-6. Based on these findings, Dr. Oyasu proposed a bladder carcinogenesis model in which an environmental agent alters the normal cell population within the bladder, resulting in cell initiation. Subsequent production of reactive oxygen species and inflammatory cytokines both fosters transformation and promotes growth of mutant clones leading to cancer.

[2] The abbreviations used are: LEC, Long-Evans Cinnamon; MNU, methylnitrosourea; IL, interleukin; TNF, tumor necrosis factor; TGF, transforming growth factor; EGF, epidermal growth factor; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; ROI, reactive oxygen intermediate; iNOS, inducible nitric oxide synthase; 8ohg, 8-hydroxyguanine; PGE_2, prostaglandin E_2; AA, arachidonic acid.

Role of Inflammatory Cytokines in Carcinogenesis

Dr. R. S. Kerbel (Sunnybrook Health Science Center, Toronto, Canada) opened the session on inflammatory cytokines in carcinogenesis. He introduced at least three ways in which inflammatory cytokines affect patients with cancer: (*a*) by inhibiting or enhancing tumor growth; (*b*) by causing cachexia; and (*c*) by altering cell sensitivity to chemotherapy. He proceeded to present information on the first of these, using melanoma as an example. He described the phenomena of multicytokine resistance and cytokine switching. The former is illustrated by the difference in cytokine response in early- and advanced-stage melanoma cells. Growth of early-stage melanoma cell lines was found to be inhibited by the presence of fibroblasts or inflammatory cytokines, including TNF-α, TGF-β, IL-1, and IL-6. In contrast, growth of advanced-stage melanoma cells was not inhibited by fibroblasts or the presence of cytokines and could not be stimulated in some cases. Thus, with multicytokine resistance, advanced-stage tumors have developed resistance to normally inhibitory agents. In fact, IL-6 enhanced advanced-stage melanoma cell growth *in vivo*. Moreover, in an example of cytokine switching, these cells both produce IL-6 and express its receptor. Thus, advanced-stage melanoma cells autostimulate growth with self-produced cytokines, providing them with a growth advantage.

Results from the laboratory of Dr. J. Hamada (Hokkaido University School of Medicine) echoed some of Dr. Kerbel's findings. His group found that low-grade mammary tumor cell (ER-1 cells), when injected s.c. into syngenic rats in the presence of an irritating substance (a plastic plate), became highly tumorigenic. This enhanced response in the inflammatory milieu was thought to be due to cytokines. When studied directly, EGF and TGF-β both augmented ER-1 tumorigenicity. EGF treatment also fostered metastasis. Enhanced tumorigenicity by EGF was only reversible if treatment was given for less than 24 h; with longer treatment, high-level tumorigenicity became a permanent cell characteristic.

Dr. F. Robertson (Ohio State University, Columbus, OH) elegantly expanded on the carcinogenic properties of cytokines, using a skin cancer model in Sencar mice. Using a multistage tumorigenesis protocol, she topically applied a single dose of 7,12-dimethylbenz(*a*)anthracene to the dorsal epidermis, followed by twice weekly topical treatments with 2 μg of TPA for 25 weeks. This induced infiltration of leukocytes into the dermis and stimulated epidermal hyperplasia. Dermal leukocytes produced ROIs, expressed iNOS, and produced peroxynitrite, as measured by the presence of 3-nitrotyrosine. Topical applications of TPA also induced gene expression and protein production of several pro-inflammatory cytokines, including IL-1α, TNF-α, and granulocyte macrophage colony-stimulating factor. Concomitant injection of anti-IL-1α antibodies reduced skin swelling, prevented neutrophil infiltration of neutrophils into the dermis, and inhibited epidermal hyperplasia. The most striking effect of anti-granulocyte macrophage colony-stimulating factor antibodies was the inhibition of ROI production by leukocytes. Pretreatment of mice with pentoxifylline, an inhibitor of pro-inflammatory cytokines, inhibited TPA-induced gene expression of IL-1α and TNF-α and also significantly inhibited the growth of papillomas and carcinomas at 25 weeks. Taken together, Dr. Robertson felt that these studies indicate that pro-inflammatory cytokines, in conjunction with ROIs and reactive nitrogen intermediates, may play a role in the tumor promotion process.

Whereas most work relating cytokines to malignancy focused on the up-regulation of inflammation, induction of ROIs, and alterations in cell growth, Dr. N. Taniguchi (Osaka University Medical School, Osaka, Japan) focused on how cytokines impacted the expression of antioxidant enzymes. TGF-β1, a cytokine that negatively regulates cell growth, suppressed the expression of many antioxidant enzymes in cultured hepatocytes including manganese-, zinc-, and copper-superoxide dismutases, catalase, and glutathione-*S*-transferases 1 and 2. This suppression of antioxidants correlated with increased peroxides in hepatocytes. Dr. Taniguchi proposed a model in which manganese-superoxide dismutase inhibition permitted superoxide interaction with nitric oxide to form peroxynitrite. In combination with increases in hydrogen peroxide due to the diminution of other antioxidant enzymes, this would provide a portentous opportunity for DNA damage. The high expression of TGF-β1 in hepatocellular cancer cells and possibly in colonic tumors as well supports a role for this cytokine in malignant transformation through oxidative damage.

Dr. K. Matsushima (Kanazawa University, Kanazawa, Japan) closed the session on inflammatory cytokines with a discussion of their role in cachexia. His group evaluated two adenocarcinoma cell lines, one that induced cachexia in mice and another that did not. The cell line that induced cachexia increased transcription of IL-6 mRNA at the tumor site, whereas the noncachexia cell line did not. Administration of IL-6 antibodies diminished the cachexia dramatically. Because infusion of IL-6 did not correspondingly increase cachexia, Dr. Matsushima concluded that the process was not unifactorial but required a complex of interacting factors; these fact remain to be elucidated.

Role of Oxygen Radicals in Carcinogenesis

Although many of the talks described above evaluated the role of oxygen radicals in carcinogenesis, six talks were specifically assigned to this topic. The first three of these focused on the role of nitric oxide in carcinogenesis, a rapidly growing field in carcinogenesis research. Dr. H. Ohshima (IARC, Lyon, France) reviewed the genesis of nitric oxide and how it reacts with superoxide to yield peroxynitrite, a major cause of tissue injury in inflammatory conditions. Dr. Ohshima reviewed both genetic and epigenetic consequences of peroxynitrite activity, including deamination, oxidation, and nitration. More specifically, he imparted new insights on how the p53 protein can be made nonfunctional by peroxynitrite nitration. Dr. Ohshima then described a novel assay for 8-nitroguanine, a marker for DNA damage induced by this strong oxidant. This assay has potential use as a biomarker for DNA damage in tissues and in urine.

Dr. H. Maeda (Kumamoto University School of Medicine, Kumamoto, Japan) described the role of nitric oxide formation in viral and bacterial infections. He had previously demonstrated a large outpouring of superoxide in the pulmonary tissue of mice with influenza virus infection. This was thought to be due, at least in part, to up-regulation of xanthine oxidase. He now reported excessive production of nitric oxide in the same setting, stimulated by influenza-induced production of IFN-γ. As described above, this simultaneous production of superoxide and nitric oxide would result in peroxynitrite formation. In support of this, Dr. Maeda demonstrated nitration of tyrosine residues in both pulmonary macrophages and epithelial cells. Interestingly, in all cases of fatal influenza, the virus was not found in the lung at the time of death, suggesting that the inflammatory response rather than the infection itself caused the ultimate pulmonary injury. Administration to the mice of an antioxidant (superoxide dismutase), a xanthine oxidase inhibitor (allopurinol), or an iNOS inhibitor (N^{ω}-monomethyl-L-arginine) resulted in improved influenza survival. Dr. Maeda reported similar results in terms of inflammation and tyrosine nitra-

tion in the livers of mice infected with *Salmonella typhimurium*. In the case of hepatic *S. typhimurium* infection, however, antioxidants and iNOS inhibitors resulted in increased bacterial survival in conjunction with decreased inflammatory response.

Because of the great interest in nitric oxide and peroxynitrite stimulated by the above discussions, Dr. M. K. Shigenaga's presentation on methods to detect 3-nitrotyrosine, an adduct formed via peroxynitrite nitration, was aptly timed. He reported a tandem high-performance liquid chromatography-EC/UV assay for detection of 3-nitrotyrosine in tissue. Macrophages and microglial cells activated by lipopolysaccharide and IFN-γ demonstrated high levels of this adduct; moreover, N^{ω}-monomethyl-L-arginine inhibited its formation. In the clinical setting, Dr. Shigenaga reported higher levels of 3-nitrotyrosine in tissue from persons with inflammatory conditions than in persons without those conditions. He suggested that this assay might be a useful tool for monitoring interventions against the damage caused by reactive nitrogen oxide species.

Dr. F. Okada (Hokkaido University) switched the theme of the remaining portion of this session to studies of oxidative damage using 8ohg as a marker for oxidative stress. In a model of syngeneic mice, he reported that weakly tumorigenic fibrosarcoma cells became considerably more aggressive if coimplanted with a foreign body that induced inflammation (either a geletin sponge or a plastic plate). These tumor cells developed a stable malignant phenotype that remained aggressive when passaged into other mice, even in the absence of a foreign body stimulant. One distinct difference between the aggressive tumor cells and their weakly tumorigenic parent cells was increased PGE_2 production by the former. Dr. Okada speculated that heightened PGE_2 production might facilitate the ability of the malignant clone to evade immune detection. Simultaneous local induction of antioxidative enzyme and antioxidative protein limited tumor progression *in vivo*; it also diminished 8ohg levels. Dr. Okada concluded that oxidative stress was an important cause of tumor progression.

Dr. S. Okada (Okayama University, Okayama, Japan) expanded on the topic of oxygen radicals by stressing the importance of transition metals in sustaining free radical reactions via Fenton and Fenton-like reactions. He particularly focused on iron that, by converting less-reactive radicals to highly reactive ones, can lead to severe oxidant damage. The i.p. injection into rats and mice of two different iron complexes (ferric nitrilotriacetate and ethylenediaminediacetate) resulted in profound lipid peroxidation in the kidney and acute tubular necrosis. This tissue necrosis was thought to release even more free iron, accelerating the damage. With repeated injections, kidney tumors resulted. Formation of 8ohg was evident in renal tissue as early as 1 h after injection. Vitamin E seemed to limit oxidative damage as evinced by 8ohg levels and also by decreased tumor formation. Dr. Okada concluded that iron complexes, even in small amounts, can cause free radical reactions that may be detrimental to the host.

Dr. H. Kasai (University of Occupational and Environmental Health, Kitakyushu, Japan) concluded the session on oxygen radicals by discussing evaluations of 8ohg in diseased human tissue and in rodent models. He observed high levels of 8ohg in liver tissue from patients with chronic hepatitis, in stomach tissue from patients with *H. pylori* infection, and in lung tissue from patients with pulmonary malignancy. In a hamster model, intratracheal asbestos augmented lung 8ohg levels, as did the chronic administration of ethanol to rats (elevated 8ohg in liver, esophagus, and pancreas). In other work from Dr. Kasai's laboratory, leukocyte 8ohg levels were found to be substantially higher in smokers than in nonsmokers. Despite the wide variability in 8ohg levels between individuals, a dose-response relationship was observed between this adduct and the quantity of cigarettes smoked/day. Dr. Kasai also reported on a novel endonuclease nicking assay to detect repair of 8ohg lesions. In general, he found that repair levels paralleled the levels of damage.

Inhibition of Carcinogenesis by Anti-Inflammatory Agents

Numerous studies have been undertaken in animals to interrupt the progression of neoplasia from the preinvasive stage associated with chronic inflammation to the stage of invasive malignancy. Dr. C. W. Boone (National Cancer Institute, Bethesda, MD) broached this topic with an overview of the National Cancer Institute's program to use antioxidants in clinical chemoprevention trials. After screening over 2500 agents in animals, the Chemoprevention Branch identified agents within several classes of antioxidant agents that merited further investigation, including certain tannins, phenylpropanoids, flavonoids, retinoids, and organic sulfur compounds. These agents are thought either to scavenge oxygen radicals or electrophiles or to inhibit AA metabolism. Over 35 human trials have been implemented testing these compounds.

Dr. Boone was followed by Dr. H. Mukhtar (Case Western Research University, Cleveland, OH) who presented a fascinating talk on the use of green tea and ginger extracts to prevent skin carcinogenesis. Green tea and ginger have been epidemiologically linked to cancer protection. In a mouse model, skin application of a polyphenolic fraction prepared from green tea substantially protected against skin tumors normally caused by tumor promoters or in initiated skin by repeated UV radiation exposures. This corresponded with marked reductions in epidermal ornithine decarboxylase, cyclooxygenase, and lipoxygenase activity as well as epidermal edema and hyperplasia. Moreover, a protective effect was found in all stages of tumorigenesis: initiation, promotion, and progression. Similar findings were reported for ginger extracts. Preliminary studies in human volunteers strongly supported a protective effect of tea polyphenols against UV radiation.

Dr. K. Tazawa (Toyama Medical and Pharmaceutical University, Toyama, Japan) proposed a novel method to limit azoxymethane-induced colon cancer in the rat. Following the hypothesis that bacterial enzymes contribute to malignancy by forming carcinogenic metabolites, his group found that dietary supplementation with 20% apple pectin markedly decreased the formation of colonic tumors. Biochemically, apple pectin, a methoxylated polymer of galacturonic acid that has bacteriostatic activity, lowered colonic PGE_2 production in a dose-dependent fashion, decreased fecal β-glucosidase and azoreductase, and pared fecal β-glucuronidase activity. Also, 20% apple pectin significantly inhibited the incidence of the experimental portal bacteremia with methotrexate and reduced the incidence of hepatic metastasis in a rat model. The loss of enzyme activity may indicate decreased ability of the colonic flora to metabolize procarcinogens from tumor promoters.

Dr. Y. Konishi (Nara Medical University, Nara, Japan) followed this discussion with a presentation of the effects of anti-inflammatory agents on liver damage in rats fed a choline-deficient, L-amino acid-defined diet. This diet, which causes accumulation of free AA as well as 1,2-*sn*-diradylglycerol in the liver, has been hypothesized to enhance tumorigenesis by causing chronic activation of protein kinase C. However, the role of the accumulated free AA in the pathological lesion induction (including hepatocarcinogenesis) has never been studied. His group has focused on this point. The cyclooxygenase inhibitors aspirin and piroxicam decreased the development of cirrhosis and preneoplastic lesions and the formation of 8ohg adducts. The phopholipase-A_2 inhibitor, p-bromophenacylbromide was far less potent but exerted similar effects.

Interestingly, however, lipoxygenase inhibitors (quercetin and nodihydroguaiaretic acid) decreased tumor formation but had no impact on formation of oxidative adducts or development of cirrhosis. This controverts the precept that 8ohg is a strong marker for carcinogenesis and warrants further investigation.

The session on chemoprevention was concluded by Dr. J. Hamuro (Ajinomoto Co. Inc., Kawasaki, Japan) who presented one of the few positive perspectives on the inflammatory process presented at this meeting. His group discovered that coadministration of lentinan, a polysaccharide that inhibits viral and chemical carcinogenesis, with IL-2 resulted in increased neutrophils, macrophages, and T lymphocytes surrounding transplanted tumor tissue in the mouse; complete cure of the malignancy resulted, with the coappearance of reticular fibers and organ-specific types of wound healing. Lentinan also prevented conversion of reductive monocytes into reactive, oxidative monocytes. Because these activated cells release the Th2 cytokines that promote cachexia and tumor progression, Dr. Hamuro speculated that polysaccharides such as lentinan may play a role in primary and secondary cancer prevention, as well as in cancer progression.

Summary and Recommendations for Future Study

Participants in this meeting uniformly agreed that chronic inflammation, whether from infection, environmental exposures, or endogenous processes, greatly increases the risk for cancer in affected organs. One of the principal mechanisms imputed was production of ROIs and nitrogen intermediates. These free radicals adversely impact proteins, lipid membranes, and DNA. Inflammatory cytokines modify the process, either up- or down-regulating production of reactive intermediates. They also induce leukocyte chemotaxis and modify cell growth and proliferation, potentially enhancing cancer promotion and progression. Biochemical markers are being widely developed to characterize free radical damage and evaluate the effects of putative carcinogens and chemopreventive agents. Animal models of carcinogenesis have already yielded significant information on cancer prevention strategies. These various therapies act at many steps of the inflammatory cascade, providing great optimism that synergistic, anti-inflammatory chemoprevention is a possibility in the future.

Most of the presentations focused on the adverse consequences of the inflammatory response. It was conceded, however, that inflammation, by ridding the body of foreign invaders, has an important purpose. Consequently, modulating the inflammatory response to prevent cancer may adversely impact our capacity to combat infectious diseases. Moreover, it is apparent that although the inflammatory response may in some settings increase the likelihood of cancer, in other scenarios, the inflammatory response destroys malignant cells. Will chemoprevention strategies suggested in animal models subvert the immune response to the detriment of the host? Can we augment good inflammatory responses while selectively suppressing others? This type of fine tuning requires a much better understanding of the inflammation-to-malignancy pathway.

Much of the work presented at this symposium relied on animal models. Translation of this work into humans is a complex, expensive, and potentially dangerous enterprise. Putative biomarkers put forth to evaluate therapeutic outcomes are not yet sufficiently tested in humans to be useful. The prospect of clinical trials is all the more daunting given this lack of intermediate markers. Moreover, few of the chemopreventive agents mentioned in this symposium have even been tested for safety in humans. Although understanding the mechanisms of disease in animals is a valid enterprise in its own right, the ultimate success is to translate this understanding to prevention or relief of human suffering. From a more mundane standpoint, funding for cancer research comes from people banking on its practical application. The fruits of the basic research presented at this symposium are yet to be realized. It was the belief of those at the Sapporo conference, however, that multidisciplinary seminars such as this plant the seeds for collaborative ideas and technologies that will ultimately enhance our ability to prevent malignancy.

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