Infection, Cancer and Prevention: Report of the 19th International Symposium of the Foundation for Promotion of Cancer Research

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INTRODUCTION

‘Infection, Cancer and Prevention’, the Nineteenth International Symposium of the Foundation for Promotion of Cancer Research, was held in Tokyo on 21–23 February 2006. The symposium was organized by Drs Daizo Saito, Martin Blaser, Tadao Kakizoe, Kumao Toyoshima, Hiroshi Yoshikura, Toshiya Hirayama, Tadahito Kanda, Kunitada Shimotohno and Kensei Tobinai, with Dr Takashi Sugimura as advisor.

OPENING ADDRESSES

Professor Takashi Sugimura (National Cancer Center, Tokyo) opened the symposium with a welcome address and a review of previous symposia (1,2). The Foundation for Promotion of Cancer Research has held this International Symposium annually since 1987, as one of the activities of the first 10-year strategy for its cancer control program, and has continued through the second and third terms of the strategy. From the first to the 18th symposium, the total number of invited speakers was 578: 307 from Japan, 183 from the USA, 17 from the UK, 14 from France and 57 from 17 other nations. For this year’s symposium, the Foundation invited 10 speakers from abroad and 24 from Japan (Fig. 1). Professor Sugimura stated his personal interest in this year’s topic and emphasized that the content of the program was divided into three main topics: infectious route, cancer mechanism and prevention. Next, the international chairman Professor Martin Blaser (New York University) after welcoming all attendees summarized the microbes implicated in the causation of human cancers. During his talk, Professor Blaser reported that cancers could be due to the loss of indigenous microbes, and summarized the mechanisms by which their loss could enhance disease risk: direct effects on host cell physiology, loss of suppression of other endogenous organisms and ease of colonization by acquired organisms.

INFECTION

VIRUS

Overview

In contrast to other types of carcinogenesis, virus-induced carcinogenesis is characterized by its clear etiology: persistent viral infection. Therefore, prevention or cure of infection can prevent development of cancer. Dr Hiroshi Yoshikura (Ministry of Health, Labour and Welfare, Japan) in his talk ‘Virus-related carcinogenesis’, reviewed the interaction modes between virus and host. Viruses are implicated in various cancers including HPV in cervical cancer, HBV and HCV in liver cancer, HTLV-1 in adult T-cell leukemia and EBV in certain types of lymphoma and in gastric cancer. The modes of interaction differ from virus to virus and from tissue to tissue. EBV is known to be involved in lymphoma and possibly in gastric cancer. HBV and HCV are responsible for liver cancer but the pathological processes leading to liver cancer appear different. He also emphasized that hallmarks of cancer occur through multi-step processes, which can be genetic or epigenetic. Thus, the virus may contribute not to the whole but only to part of the steps, either in an early stage of initiation, or in later stages of promotion when the initiation events already have taken place. Genotypes of virus and those of the hosts, environment of the target tissues, developmental stages of the target tissues all may influence the mode of the interaction.

Epstein–Barr Virus (EBV)

EBV infection alone is believed to be sufficient for inducing lymphoproliferative disorders. Professor Kenzo Takada
(Hokkaido University, Japan) reported that virus activation occurs when EBV-carrying cells differentiate to antibody-producing plasma cells following exposure of B cell antigen receptor (BCR) to its cognate antigen. He described the unique Akata cell system in which the viral lytic cycle can be efficiently induced by treatment with anti-Ig antibodies. By using this system, it was demonstrated that the BZLF1 gene provides the switch from a latent to a lytic replicative cycle. The BZLF1 promoter is a target of BCR signaling, and PI3K is a determinant of responsiveness to BCR-mediated EBV activation (3). Clinically, proliferation of EBV-infected B cells are more likely to take place in immunocompromised hosts such as transplant recipients. This condition is termed post-transplant lymphoproliferative disease (PTLD) and is one of the important complications that determine prognosis of transplant patients.

HBV AND HCV

Professor Hiroshi Yoshizawa (Hiroshima University, Japan) reported preliminary results of the 5-year National project for the management of viral hepatitis and the eventual prevention of HCC in those aged over 40 years. The mortality resulting from hepatocellular carcinoma (HCC) has been increasing since 1975. In fiscal year 2002, this number reached 34 637, which was the third highest for deaths caused by malignant neoplasms, after the 63 405 deaths from lung cancer and 49 213 from stomach cancer. Approximately 81% of HCC cases are caused by persistent infections by hepatitis C virus (HCV), ~13% by hepatitis B virus (HBV), and the remaining 6% are of unknown etiology. A large-scale sero-epidemiological survey of HBV and HCV infections among 3 485 648 first-time blood donors at Japanese Red Cross Blood Centers from 1995 to 2000 has identified 968 000 HBV carriers and 885 000 HCV carriers in the 16–69 age groups and that 73.8% of HBV carriers (714 000) and 85.8% of HCV carriers (759 000) are concentrated in age groups over 40 years, for whom the risk of developing HCC is increased. To solve this problem, the Japanese Government launched a 5-year national project in April 2002 aimed at the management of viral hepatitis and the eventual prevention of HCC in those aged over 40 years. For fiscal years 2002–2004, 5 408 172 individuals received screening tests for HBV and 65 704 (1.2%) were found to be...
positive for HBsAg (HBV carriers). Of the 5372501 individuals who received the HCV screening test, 71715 (1.3%) were found to be HCV carriers.

Dr Miriam Alter (Centers for Disease Control and Prevention, Atlanta, USA) reported that even in countries with substantial resources, most high-risk adults remain unvaccinated, risk-reduction services are lacking and health-care-related HBV and HCV infections continue to occur. Programs on HBV for infants and adolescents in these countries have reduced new infection rates among children by >90%. Relatively few adults have been vaccinated and high-risk sexual activity and injection drug use continue to account for most cases of newly acquired hepatitis B (4).

The major risk factors associated with acquiring HCV infection include unsafe therapeutic injections, transfusions from unscreened donors and injection drug use. In countries with high or moderate HCV prevalence, unsafe therapeutic injections appear to be the predominant mode of HCV transmission and may account for up to 40% of all HCV infections worldwide. In most of the low prevalence areas, illegal injection drug use is the predominant mode of transmission. In these countries, transfusion- and transplant-associated HCV infections have been virtually eliminated through routine testing of donors and declines in cases among drug users have also been observed (5). Both HBV and HCV infections can be prevented by screening donors, virus inactivation of plasma-derived products, risk-reduction counseling and services, and implementation and maintenance of infection control practices. For HBV, however, the single most effective prevention measure is immunization. Despite the availability of an effective vaccine for more than 20 years, unfortunately most of the world’s children remain at risk for HBV infection.

**HTLV-I**

Professor Masao Matsuoka (Kyoto University, Japan) reviewed the transmission and natural course of HTLV-I Infection. After transmission, HTLV-I induces proliferation of CD4 positive T-lymphocytes by activating accessory genes. HTLV-I encodes accessory genes in the pX region, which is present between env and 3-LTR. The tax gene plays central roles in viral gene transcription, viral replication and the proliferation of HTLV-I-infected cells. Tax enhances viral gene transcription from the 5-LTR via interaction with the cyclic AMP responsive element binding protein (CREB). Tax also interacts with cellular factors and activates transcriptional pathways, such as NF-kB, AP-1 and SRF. For example, activation of NF-kB induces transcription of cytokines and their receptor genes, as well as anti-apoptotic genes, such as bcl-xL and survivin. With these actions, HTLV-I increases the number of infected cells, which promotes its own transmission. The pleiotropic actions of accessory genes promote proliferation of HTLV-I-infected cells. Such strategies to increase the number of infected cells facilitate transmission of HTLV-I. Because the HTLV-I provirus is randomly integrated into the host genome, the identification of integration sites enables identification of each infected clone and to trace the kinetics of infected cells in vivo. Analyses using inverse PCR, which can identify the integration sites of the HTLV-I provirus, revealed that the proliferation of infected cells is oligoclonal and that infected cells persistently survive in vivo. Importantly, in carriers, such clonal expansion is directly associated with the onset of ATL. Thus, the viral strategies to increase the number of HTLV-I-infected cells work efficiently in most carriers without any adverse effects.

**Human Papilloma Virus (HPV)**

Professor Magnus von Knebel Doeberitz (University of Heidelberg, Germany) reported his research on human papilloma virus (HPV) and cancer in the lower female genital tract. HPV-related carcinogenesis is the rare consequence of aberrant, deregulated (high risk) HR-HPV infections of reserve cells of the cervical transformation zone in the lower genital tract. There are 15 types of HPV associated with malignant transformation of epithelial cells of the lower genital tract. These viruses encode two viral genes (E6 and E7) that confer numerous oncogenic features to normal replicating cells. In contrast to the normal life cycle of the HPV, the aberrant and deregulated expression of the E6 and E7 genes in the basal or para-basal cells of the epithelium provoke chromosomal instability, significantly enhanced recombination of chromosome fragments and eventually integration of fragments of the viral genome. This results in a strong over-expression of the cyclin-dependent kinase inhibitor p16INK4a, that can thus be used as a very specific and sensitive biomarker for HPV-transformed cells or tissues in histological sections, cytological, or biochemical samples. Because integration of the viral genomes creates specific molecular fingerprints for each individual HPV-transformed cell clone, analysis of the integration sites allows monitoring of the clonal origin of individual dysplastic lesions (6). This research revealed that the vast majority of HPV-induced dysplastic lesions or cancers in the lower female genital tract were derived from initially transformed reserve cells of the transformation zone that subsequently spread to more distant sites in the cervix, vagina, or vulva. Biomarkers such as p16INK4a that could be delineated by the analysis of the molecular pathways involved in HPV-induced carcinogenesis permit the design of novel preventive, diagnostic and eventually also therapeutic strategies to combat this widespread disease.

A cohort study to identify the determining factors for CIN regression/progression of cervical precursor lesions was presented by Professor Hiroyuki Yoshikawa (University of Tsukuba, Japan). In this study, Dr Yoshikawa defined CIN progression as progression to CIN III and CIN regression as normal colposcopy and at least two consecutive negative cervical smears. CIN II lesions were less likely to regress than...
CIN I lesions (2 years regression rate, 47% versus 59%, $P = 0.02$), and more likely to progress to CIN III than CIN I lesions (overall progression rate, 26% versus 10%, $P = 0.01$). Furthermore, detection of high-risk HPVs (HPV16/18/31/33/35/52/58) was strongly associated with both persistence ($P < 0.001$) and progression ($P = 0.007$) of CIN lesions. In younger women, the regression rate was higher ($P < 0.001$) and the progression rate was lower ($P = 0.03$). After adjustment for CIN grade, HPV types and age, environmental factors such as cigarette smoking, oral contraceptive use, lifetime number of sexual partners and Chlamydia trachomatis infection significantly reduced the rate of CIN regression ($P < 0.05$), but did not promote progression. Interestingly, HLA class II DRB1*1302 allele exerted a protective effect against CIN progression ($P = 0.01$), but did not contribute to regression. In this study, parity did not correlate with either regression or progression of CIN I/II lesions. These data suggested that HPV type is the strongest determinant of both regression and progression of CIN, while host environmental factors may correlate with CIN regression and host genetic variation may be associated with CIN progression.

**Kaposi’s Sarcoma Herpes Virus (KSHV)**

Professor Thomas Schulz (Hannover Medical School, Germany) reviewed the pleiotropic effects of KSHV, also known as human herpes virus 8 (HHV8). As an infectious agent, KSHV is an essential factor in the pathogenesis of Kaposi’s sarcoma (KS), multi-centric Castleman’s disease (MCD) and primary effusion lymphoma (PEL). Case reports suggest occasional involvement in bone marrow hypoplasia and haemophagocytic syndrome, but other disease associations are unconfirmed or controversial. KSHV has co-evolved with human populations over many thousands of years and is related to recently discovered rhadinoviruses in old world primates. KSHV establishes a latent infection in the majority of infected cells in KS, MCD and PEL but lytic replication occurs in a small fraction of infected cells. Importantly, KSHV-associated disease is of particular importance in immunosuppressed individuals, and in particular, in patients with HIV infection and transplant recipients.

**Parasite**

A picture of schistosome-induced carcinogenesis was presented by Professor Nobuo Ohta (Tokyo Medical and Dental University, Japan). Three major Schistosoma species are known as human parasites: *S. japonicum*, *S. mansoni* and *S. haematobium*. Two main approaches have been taken to elucidate the enigma relating parasite infection and carcinogenesis. One direct avenue is to seek mutagenic activities in the parasite components. In the case of *S. japonicum*, the Ames test was applied to detect mutagenic activities both in adult worms and ova, however, no evidence of mutagenic activity was obtained. A similar test was reported using the umu-test and V79/HGPRT gene mutation assay, but there again was little or no positive activity in the parasite materials. Those results suggest that components of the schistosome parasite themselves do not contain mutagenic activities involved in the carcinogenesis. Studies on chromosomal aberrations in bladder cancer cases in Egypt indicated that no particular profiles were detected between cases with and without *S. haematobium* infection. The other approach discussed is testing of the immunomodulatory effects by schistosome infections. It is well known that helminthic infections, including schistosomiasis, induce host immune responses that are polarized to Th2-dominance. Therefore, it might be possible to assume that schistosome infection inhibits host immune responses responsible for eliminating tumor cells, because tumor immunity often involves Th1 responses that are inhibited in Th2-skewed environments. Immunomodulation during schistosome infection also might induce altered susceptibility to other viral and/or bacterial infections, some of which are carcinogenic.

**Bacteria**

*Helicobacter pylori* provides a significant model system for microbial–host interactions leading to oncogenesis. Professor Martin Blaser (New York University, USA) addressed this interesting issue. There are far more bacterial cells living in the human body than there are human cells. Although our interactions with our microbes are generally peaceful, biological costs (i.e. disease) are associated with this carriage. *H. pylori* is an organism that persists in the stomach of a human for essentially its entire lifetime. Phylogenetic studies indicate that *H. pylori* and its ancestors have long colonized humans and our ancestors (7). This long co-habitation has selected for *H. pylori* strains that can signal the host to maximize colonization efficiency. *H. pylori* persistence is achieved by cross-signaling between microbe and host that is linked in a negative-feedback loop (8). Mathematical modeling indicates that such a circuit is the only way to achieve persistence. Within such a model, microbially induced mortality late in life (e.g. owing to cancer) is not selected against; in fact, there may be positive selection for such a phenomenon within social groups. Adaptations that allow *H. pylori* to persist include having a surface with low toxicity to the host. The low endotoxicity of its lipopolysaccharide and its Lewis antigen expression are analogous to the surface expression of Lewisx on the surface of schistosomes and their ova. *H. pylori* can regulate its local environment by producing signals, such as the CagA protein, that interact with the host epithelium. One consequence of the interaction is a microbial effect on host cytokines and on local (gastric) hormones, which has effects related to both physiologic processes and disease.
CANCER MECHANISM

FREE RADICALS

Endogenously formed free radicals facilitate accelerated mutational damage directly and indirectly at the site of inflammation, and formation of nitroguanosine per se leads to DNA/RNA damage via generation of free radicals. Professor Hiroshi Maeda (Sojo University, Japan) reviewed the most common agents involved in the classic theory of carcinogenesis: radiation, chemicals and infection. Influenza and Sendai virus infections in mice induce enormous bursts of superoxide anion (O$_2^-$) in the lung, at levels more than 200-fold that of uninfected control lung. This O$_2^-$ was not necessarily derived from macrophages, but from activated xanthine oxidase. Furthermore, parallel production of nitric oxide (NO) and O$_2^-$ was found. ONOO$^-$ is a highly oxidizing and nitrating agent to both proteins and nucleic acids. As a consequence, the mutant virus increased about seven- to eight-fold during one disease life span of mice in vivo (7–10 days). This indicates that endogenously formed ONOO$^-$ is an endogenous mutagen. Furthermore, nitrosoguanine thus formed can generate superoxide by NO synthase and cytochrome P450 reductase, and thus accelerate free radical generation via formation of nitroguanosine. In separate experiments, Dr Maeda has reported that heterocyclic amines become the catalyst to generate superoxide radicals using cytochrome P450 reductase or cytochrome b5 reductase (9). Also, it was demonstrated that chemical carcinogens applied topically induced iNOS. These results indicate that chemical carcinogenesis endogenously activates free radical generation in one of several ways. Dr Maeda concluded that, common denominators in infection/inflammation, chemical-induced oncogenesis and radiation are the free radicals generated in situ. The importance of free radical scavengers as a counter measure against such insults can be envisaged in addition to antimicrobial therapy against infection.

VIRUS

EPSTEIN–BARR VIRUS (EBV)

EBV-encoded non-coding RNAs (EBERs) are more important than thus far considered. Professor Kenzo Takada (Hokkaido University, Japan) reviewed this exciting issue. EBERs, consisting of EBER1 and EBER2, are non-polyadenylated RNAs transcribed by the RNA polymerase III system. They are 166 and 172 nucleotides long, respectively, and the most abundant EBV RNAs are in latently infected cells. Most EBERs localize to the cell nucleus, where they are complexed with cellular La protein, which is recognized by specific antisera from patients with systemic lupus erythematousus. EBERs show striking similarity in secondary structures with adenovirus VA1 and VA2, and U6 cell small RNAs, which also complex with the La protein, although the significance of their interactions is not known. Furthermore, as with VA1, EBERs bind double-stranded RNA-activated protein kinase (PKR) and inhibit its phosphorylation in a cell-free system. EBERs confer resistance to interferon-induced or FAS ligand-mediated apoptosis in both lymphoid and epithelioid cells by directly binding to PKR and inhibiting its phosphorylation. Alternatively, EBERs induce expression of cellular growth factors, i.e. interleukin-10 in B cells, interleukin-9 in T cells, and insulin-like growth factor-1 in epithelial cells, each of which acts as an autocrine growth factor. Furthermore, EBERs are important for growth transformation of peripheral B cells by EBV (10). The transforming efficiency of EBER-knockout EBV is approximately 100-fold less than that of wild-type EBV, and an EBER-knock-in restores the transforming ability.

Professor Katsuyuki Aozasa (Osaka University Japan) reported a distinctive type of lymphoma strongly associated with EBV Infection, the pyothorax-associated lymphoma (PAL). This disease was originally described by Dr Aozasa as a distinctive clinicopathologic entity in 1987, and now is listed as a disease entity in the WHO Classification of Tumors (11). The most common symptoms when patients are admitted to the hospital are chest pain and fever. Serum neuron-specific enolase level is occasionally elevated, suggesting a diagnosis of small cell lung cancer. Histologically, PAL usually shows diffuse proliferation of large cells of B-cell type, diffuse large B-cell lymphoma (DLBL). The gene expression profile of PAL is distinct from nodal DLBL in its higher expression level of interferon-inducible genes. PAL is strongly associated with Epstein–Barr virus (EBV) infection with expression of EBV latent genes $\text{EBNA-2}^+$ and/or $\text{LMP-1}^+$ together with $\text{EBNA-1}^+$, $\text{EBNA-2}^+$, and $\text{LMP-1}^+$, the same pattern observed in opportunistic lymphomas such as lymphomas developing in AIDS and organ transplant recipients. Taken together, PAL is a distinct entity, both in its clinicopathologic presentation as well as its gene expression profile.

HBV AND HCV

Dr Christian Bréchot (Institut National de la Santé et de la Recherche Médicale, France) reported his in vivo and in vitro studies on the direct analyses of HCCs. Interestingly, the pattern of genetic changes in hepatocellular carcinoma (HCC) cells is clearly different in HBV and HCV-related HCCs. Studies based on micro- and macro-array technologies also have shown a clearly different pattern of gene expression in HBV and HCV-linked HCCs. A large number of investigations have provided support for these findings. They have shown that both HBV and HCV proteins are capable of interfering with a number of major signaling pathways controlling cell proliferation, viability and important metabolic networks. In addition, integration of HBV DNA into the host genome contributes to the deregulation of
several key events in cellular gene expression. Dr Bréchot has been tackling these issues by combining in vivo studies, based on the direct analyses of HCCs, and experimental in vitro and in vivo models demonstrating: (i) integration of HBV DNA into or in the vicinity of cellular genes controlling major metabolic pathways is, in contrast with current views, a frequent finding; in particular, hot spots for HBV DNA insertion into the human telomerase and calcium homeostasis-regulator genes have been identified; (ii) HCV core protein, encoded by HCV natural variants isolated from HCC cells, down-regulates TGF-β dependent signaling, which is a key factor for cell viability and proliferation control. These results show that this property is specifically related to tumor and non-tumor-derived HCV cores. These findings support the concept of an important biological impact of the mutations shown in HCC-derived HCV core sequences.

Thereafter, Professor Kunitada Shimotono (Kyoto University, Japan) discussed an exciting model of viral replication and reviewed the viral and cellular factors involved in host/HCV interaction. A major impediment to the study of the complete life cycle of HCV has been the lack of robust model systems. However, a cell culture system that is able to sustain replication of the viral genome has been established. In this system, a subgenomic HCV RNA (replicon) in which the structural protein coding region has been replaced by a selection marker, replicates robustly in Huh7, a human hepatoma-derived cell line. The development of the subgenomic replicon system has contributed to the understanding of viral replication and virus-cell interactions, and, furthermore, provides a means to test therapeutic targets. Using this system, the effects of various compounds on HCV replication were examined. It was found that cyclosporin A (CsA), an immunosuppressant, has a suppressive effect on replication of the HCV genome. CsA also inhibited replication of the HCV genome in a cultured human hepatocyte cell line infected with HCV-positive plasma. The anti-HCV activity of CsA appeared to be independent of its immunosuppressive function. In fact, NIM811, a CsA derivative with no immunosuppressive activity, showed similar anti-HCV activity. To further clarify the role of CsA in suppression of HCV replication, Shimotono and colleagues also analyzed cellular functions that are modified by CsA, and found that cyclophilin B (CypB), a cellular target of CsA, positively regulated HCV replication.

Professor Kazuhiko Koike (University of Tokyo, Japan) reviewed the experimental evidence for the contribution of hepatitis C virus (HCV) on the development of insulin resistance in patients with HCV infection. In patients with chronic hepatitis C, a significant decrease in the serum levels of total cholesterol and apolipoproteins C2 and C3 was observed, compared to those with chronic hepatitis B who were comparable in liver function. In an animal model, C18:1 mono-unsaturated fatty acids were significantly increased in the liver in mice transgenic HCV core gene, similarly to that observed in the liver from human hepatitis C patients. Thus, a disturbance in lipid metabolism was observed in both humans and in an HCV mouse model, supporting that it is a specific event in HCV infection. A significant increase in the value of an indicator for insulin resistance, HOMAIR, was observed in patients with chronic hepatitis C, even at the very early stages of chronic hepatitis. In the animal model, marked insulin resistance was exhibited from a very young age in the HCV core gene transgenic mice; the insulin resistance was chiefly due to deficient insulin action on the suppression of glucose production in the liver. Thus, ability of insulin to lower plasma glucose levels in the HCV transgenic mice was impaired, as observed in chronic hepatitis C patients. These results provide direct experimental evidence for the contribution of HCV to the development of insulin resistance in human HCV infection that ultimately leads to the development of type 2 diabetes.

**HTLV-1**

Molecular mechanisms of leukemogenesis in HTLV-I-induced adult T-cell leukemia were reviewed by Professor Masao Matsuoka (Kyoto University, Japan). The tax gene, which is one of the accessory genes encoded by human T-cell leukemia virus type I (HTLV-I), plays a critical role in the proliferation of HTLV-I-infected cells by its pleiotropic actions. However, since tax protein is the major target of cytotoxic T-lymphocytes (CTLs), Tax-expressing cells are rapidly eliminated in vivo. Thus, tax expression confers both advantages and disadvantages to HTLV-I-infected cells. In HTLV-I-infected cells, Rex, p30 and HBZ suppress tax expression. In addition, loss of tax expression is frequently observed in leukemic cells. Among fresh leukemic cells isolated from ATL patients, about 60% of cases do not express the tax gene transcript. Interestingly, ATL cells with tax gene changes expressed tax transcripts, suggesting that ATL cells do not silence transcription when the tax gene is abortive.

**Human Papilloma Virus (HPV)**

Professor Paul Lambert (University of Wisconsin) reviewed the multiple properties of the Human Papilloma Virus (HPV) E7 oncogene and its relation to cervical cancer. High-risk human papilloma viruses encode two oncogenes, E6 and E7, expressed in nearly all cervical cancers. E7 is a multi-functional protein in vitro and induces multiple phenotypes in transgenic mice, including cervical cancers when the mice are treated with estrogen. The molecular mechanisms of E7 function have been studied in vitro or in cutaneous epithelium in transgenic mice for many years, with conflicting results about the importance of pRb inactivation by E7 versus the importance of the effects of E7 on other targets. However, little work has been done in cervical tissue in vivo. In genetically altered mice, E7 expression in estrogen-treated murine cervix-induced dysplasia and invasive cancers, however, targeted Rb inactivation in cervical
epithelium was not sufficient to induce any cervical dysplasia or neoplasia. Furthermore, E7-induced cervical cancer formation even when the E7-pRb interaction was disrupted by the use of a knock-in mouse carrying an E7-resistant mutant Rb allele. These data indicate that non-pRb targets of E7 play critical roles in cervical carcinogenesis.

Roles of the HPV E6 protein on the development of cervical cancer were presented by Dr Tohru Kiyono (National Cancer Center Research Institute, Japan). The E6 protein is known to inhibit differentiation of keratinocytes through unidentified mechanisms (12). Lines of evidence have shown that Notch1 is a key determinant of keratinocyte differentiation and functions as a tumor suppressor in the mammalian epidermis. In the uterine cervix, Notch1 protein levels are elevated in cervical intraepithelial neoplastic (CIN) lesions, but in turn, substantially reduced in invasive cervical carcinoma cells. However, the underlying mechanism(s) for Notch1 down regulation in cervical cancer is not known. The Notch1 gene is a novel target of p53 transcription factor, and HPV E6 down-regulates Notch1 expression through p53 degradation in normal human epithelial cells. By comparing a series of E6 mutants, the activity of E6 to inactivate p53 correlates with Notch1 repression, and knockdown of p53 by short hairpin RNA (shRNA) results in reduced Notch1 expression at the level of transcription. A p53-responsive element in the Notch1 promoter was identified. Expression of either E6 or p53-shRNA suppresses the induction of a keratinocyte-specific early differentiation marker, involucrin. Together, these results demonstrated that HPV E6 indirectly inhibits expression of another important tumor suppressor, Notch1, and through its action degrades p53.

BACTERIA

*Helicobacter pylori* is a part of human gastric physiology and pathophysiology, and as it is disappearing, disease risks are changing. Professor Martin Blaser (New York University) in his lecture ‘The epidemiology of *H. pylori* and cancer: past, present and future’ addressed this issue. A substantial body of evidence indicates that *H. pylori* has been colonizing the human stomach since our earliest origins, but it now is apparent that with industrialization and socio-economic development, *H. pylori* has been disappearing. For probably the first time in human history, large numbers of adults are *H. pylori*-negative. As a result, by comparing those who are *H. pylori*-positive or negative, we now can determine the consequences (biological costs) of *H. pylori* carriage. The data are clear that the presence of *H. pylori* increases the risk of developing adenocarcinoma of the stomach, both of the intestinal and the diffuse types as well as gastric lymphoma and peptic ulcer disease. Particular *H. pylori* subtypes (e.g. CagA-positive) are associated with enhanced risk of gastric adenocarcinoma (13). There is increasing evidence that particular host polymorphisms, related to expression of pro-inflammatory cytokines, also are important risk factors and early life family structure affects risk as well. In countries where *H. pylori* is disappearing, gastric cancer is disappearing, but adenocarcinomas of the gastro-esophageal junction and its precursors (GERD and Barrett’s esophagus) are becoming most prevalent (14). Studies in several parts of the world now show an inverse relationship between these cancers and the presence of *H. pylori*; this inverse relationship is most marked for cagA-positive strains. In total, the data suggest a reciprocal relationship between gastric and esophageal adenocarcinomas, mediated by the presence or absence of *H. pylori*, respectively, especially cag + strains. Dr Blaser also outlines the potential mechanisms for the *H. pylori* protective role in the esophagus, including direct effects on esophageal tissue and biota, and indirect effects on gastric physiology and biota.

Then, the role of *H. pylori* CagA in gastric carcinogenesis was reviewed by Professor Masanori Hatakeyama (Hokkaido University, Japan). Infection with cagA-positive *H. pylori* is associated with atrophic gastritis, peptic ulcers and gastric adenocarcinoma. The cagA gene product CagA is injected from *H. pylori* into gastric epithelial cells and undergoes tyrosine phosphorylation by Src family kinases (SFKs). Tyrosine-phosphorylated CagA specifically interacts with the SH2 domain-containing protein tyrosine phosphatase SHP-2 and activates the phosphatase activity. CagA-activated SHP-2 causes sustained Erk-MAP kinase activation as well as cell-morphological transformation that is characterized by cells with elongated shape and elevated motility (hummingbird phenotype). Deregulation of SHP-2 by CagA appears to play a key role in gastric carcinogenesis, owing to gain-of-function mutations in PTPN11, the gene encoding SHP-2, which also has been identified in a variety of human malignancies (15). The observations indicate that SHP-2 is a bona fide oncoprotein and deregulation of SHP-2 by CagA functionally mimics gain-of-function mutations of SHP-2. Dr Hatakeyama also reported that focal adhesion kinase (FAK) is a critical substrate and downstream effector of CagA activated SHP-2. When expressed in gastric epithelial cells, CagA reduced the level of FAK tyrosine phosphorylation. The decrease in tyrosine-phosphorylated FAK was due to SHP-2-mediated dephosphorylation of FAK at the activating phosphorylation sites. Co-expression of constitutively active FAK with CagA inhibited induction of the hummingbird phenotype, whereas expression of dominant-negative FAK induced an elongated cell shape characteristic of the hummingbird phenotype. These results indicated that inhibition of FAK by SHP-2 is substantially involved in the morphogenetic activity of CagA.

Afterwards, Professor James Goldenring (Vanderbilt University, USA) presented his research on SPEM, oxyntic atrophy and pre-neoplasia in the stomach. Studies in mice have led to important insights into the origin of SPEM in response to both oxyntic atrophy and *Helicobacter* infection (16). Investigations with pharmacological ablation of parietal cells with the drug DMP777 have led to an understanding that SPEM can arise in the setting of parietal cell loss, even in the absence of significant inflammatory
infiltrate. SPEM also is a prominent finding in mice infected with *H. felis* or *H. pylori*. SPEM develops in response to parietal cell loss and chronic inflammation. In addition, mice infected chronically with *H. felis* develop gastritis cystica profunda and intramucosal adenocarcinoma. These studies support a two-stage model for gastric carcinogenesis in mice, with a first stage of parietal cell loss and induction of metaplasia followed by a second stage of neoplastic transformation supported by a chronic inflammatory milieu. Recent studies have established that both SPEM and goblet cell intestinal metaplasia are relevant precursors for gastric cancer induction in humans. However, the relationships of these metaplastic lineages to each other and neoplastic pathways in humans remain obscure.

**PREVENTION**

**VIRUS**

**HBV AND HCV**

Most of the therapeutic vaccination approaches are not ready for therapeutic use in chronic hepatitis B infection and require further refinement. Dr Jake Liang (National Institute of Health, Bethesda, USA) presented a lecture on this issue. Hepatitis B vaccines have been available in the USA since 1981 (17). The current form of HBV vaccine is produced as recombinant HBsAg in yeast and is very effective in preventing all forms of transmission, with efficacy up to 95%. Universal immunization with HBV vaccine has led to a dramatic reduction in the number of new cases of HBV infection and resulted in a substantial decrease in the incidence of hepatocellular carcinoma in parts of Asia. However, despite the success of preventive vaccines against HBV, a large number of people suffer from chronic HBV infection with progressive liver disease, leading to cirrhosis and its complications, including portal hypertension, variceal bleeding, hepatic encephalopathy and liver cancer. Therapy for hepatitis B is improving, but novel targets and strategies are still needed. Improving treatment options and developing novel therapies against HBV is pivotal in reducing the morbidity and mortality associated with this disease. Because HBV persistence is thought to be the result of poor HBV-specific T-cell responses, stimulating HBV-specific T-cell responses with therapeutic vaccines has been proposed as a novel approach for HBV therapy. Several strategies including vaccination with HBV DNA, recombinant HBV proteins, and designed HBV-specific T cell epitopes have been tested, with variable results.

Modified therapeutic schemes should be more useful to reduce the risk of HCC development in chronic hepatitis C patients than the standard interferon monotherapy. This topic was addressed by Drs Norio Hayashi and Tetsuo Takehara (Osaka University, Japan). Professor Hayashi reported a large scale, retrospective cohort study of chronic hepatitis C patients. In this study, patients were classified into three groups: sustained biochemical response (SBR) (944 patients), transient biochemical response (TBR) (791 patients), and biochemical non-response (BNR) (1234 patients). During the follow-up period, 19 patients belonging to the SBR group, 54 belonging to the group, and 174 belonging to the BNR group, and 70 untreated patients developed HCC. In addition, 164 deaths from all causes were identified. Among them, there were 119 deaths from liver-related diseases; one death was observed in the SBR group, five in the TBR group, 66 in the BNR group and 47 deaths among untreated patients. Patients who received interferon therapy had a reduced risk of HCC development to a risk ratio of 0.62, compared with untreated patients. Patients belonging to the SBR group and TBR group had a lower risk of HCC development than untreated patients. The risk for HCC development does not differ between patients belonging to the BNR group and untreated ones. Interferon therapy reduced the risk of liver-related death to a risk ratio of 0.44, compared with no antiviral treatment. Considering biochemical responses to interferon therapy, the SBR and TBR groups showed lower risk of liver-related death than the untreated group, whereas the risk did not differ between the BNR and untreated groups. In summary, this important trial provided evidence that interferon therapy for chronic hepatitis C not only reduces the risk of HCC development but also improves long-term survival, especially among patients showing SBR and TBR to the therapy.

Dr Jake Liang (National Institute of Health, Bethesda, USA) on a very hopeful note reported that hepatitis C virus-like particles can induce humoral and cellular immune responses and offers a promising approach to vaccine development (18). Despite the extensive advances in research on immune responses induced by HCV, the development of an effective vaccine against HCV has had limited success due to the lack of reliable cell culture systems and small animal models for viral propagation. Numerous approaches including recombinant HCV envelope proteins, heterologous viral vectors carrying HCV antigens, and DNA immunization strategies have been pursued. An alternative approach to the design of HCV vaccine capable of inducing protective immunity is to produce HCV-like particles (HCV-LPs). Dr Liang has reported that expressing HCV structural proteins in baculovirus (insect) cell systems generates HCV-LPs that are capable of inducing humoral and cellular immune responses in mice that are protective in a surrogate HCV-vaccinia challenge model. Dr Liang has extended similar results to non-human primates and demonstrated partial protection in chimpanzees upon challenge with HCV.

**HTLV-1**

Professor Shigeru Katamine (Nagasaki University, Japan) reported outstanding results of the 18-year intervention study involving mother to child HTLV-1 transmission in Nagasaki, Japan. So far, 190 156 pregnant women in the prefecture have enrolled in the program during the 16 years since its
start (1988–2003). Based on the annual number of total births in the prefecture, the enrollment rate is estimated to be more than 80%. Among enrollees, 6,823 women (3.6%) were found to be infected. The carrier rate has recently been decreasing from 4.9% during 1988–1992 to 3.6% (1993–1997), 2.4% (1998–2002) and 1.7% (2003). Demographic, social or behavioral factors involved in this decline remain to be identified. More than 90% of the carrier mothers agreed to refrain from breastfeeding. According to a report by the study group of APP in 1998, their prospective studies indicated that the incidence of transmission among bottle-fed children, 2.7%, was about six-fold less than that among breast-fed children, 16.9%. These results finally confirmed breast milk as the major source of maternal HTLV-I transmission and indicated that refraining from breastfeeding by carrier mothers is the most effective measure to prevent transmission. It is estimated that the intervention has prevented 1,000 cases of maternal transmission and 50 future ATL cases.

**Human Papilloma Virus (HPV)**

The high costs of virus-like particle (VLP) vaccine production and distribution, the expected type-restriction of their protection, and the unlikely prospects for therapeutic efficacy will be impediments for vaccine implementation in developing countries. Dr John Schiller (National Cancer Institute, Bethesda, USA) addressed this issue. Three large, phase III, prophylactic HPV VLP trials are currently in progress. A licensed prophylactic HPV vaccine would raise a number of implementation issues. These include the general acceptance of a vaccine targeting a sexually transmitted infection, the logistics of administering a series of three injections to adolescents or pre-adolescents, and the impact of the vaccine on compliance with cervical cancer screening programs. Topical microbicides might complement, or in some instances substitute for, VLP vaccines, if they resulted in protection against anogenital infection by a broad spectrum of HPV types.

Dr Tadahito Kanda (National Institute of Infectious Diseases, Japan) presented his research on HPV high-risk types with Anti-L2 Antibody. Two new cross-neutralization epitopes in the aa 28–42 and aa 64–81 regions of HPV16 L2 were identified by characterizing antisera obtained by immunizing rabbits with synthetic peptides. The peptides had L2 sequences from aa 14–27 (14/27), 28/42, 61/75, 64/81, 107/122, or 131/144. Antibody against peptide 14/27 did not bind to the HPV16 L1/L2-capsids, suggesting that this region is not displayed on the surface of particles. The other antibodies were found to bind to the HPV16 L1/L2-capsids. Antibodies against 28/42, 61/75, 64/81 and 107/122 were found to bind to the L1/L2-capsids of HPV16 and the other HPV types tested. These antibodies neutralized the infectious pseudovirions of different types tested (HPV16, 18, 52, 58). A mixture of antibodies against 28/42 and 64/81 showed the highest neutralizing activity among the samples tested. It is likely that antibodies against 61/75 and 64/81 recognize a common epitope and antibody against 107/122 bound to the epitope were identified previously. The L2 region displayed on the surface of HPV particles probably performs an important function for viral infectivity. Yang et al. showed that the HPV16 pseudovirions with a deletion from aa 25 to 45 in L2 have dramatically reduced infectivity. Dr Kanda also reported that substitution of alanine for arginine at aa 69 or alanine for tyrosine at aa 72 of the HPV16 L2 reduced the pseudovirion’s infectivity. Recent clinical trials demonstrated that the HPV16 and 18 L1-capsids can serve as a type-specific prophylactic vaccine against HPV16 and 18 infections, respectively.

Professor Ian Frazer (University of Queensland, Australia) presented the successes and challenges in HPV immunotherapy. HPV prophylactic vaccines will significantly reduce the burden of cervical cancer, but will be ineffective for the many women already infected with HPV and at risk for developing cancer. A successful therapeutic vaccine for HPV infection would assist in preventing cervical cancer in countries without pap smear programs, and would help with management of persistent pre-malignant conditions of the anogenital epithelium, including VIN and AIN. Such a vaccine would kill HPV-infected cells by inducing cytotoxic T-cells specific for viral non-structural proteins including E2, E4, E6 and E7, and would likely be HPV-genotype-specific. Vaccines delivering these HPV antigens, including conventional protein and adjuvant systems, polynucleotidal vaccines and viral delivery vectors have proven effective in murine transplantable tumor models, and several have proven both safe and immunogenic in humans.

**Bacteria**

Dr Daizo Saito (National Cancer Center Hospital, Japan) presented the results of the Japanese interventional randomized trial of *H. pylori* (JITHP). This study was designed to clarify the relationship between *H. pylori* eradication and the endoscopic and histologic regression or progression of inflammation, mucosal atrophy and intestinal metaplasia in eradicated and non-eradicated groups. A total of 379 and 372 subjects were enrolled in the eradication and non-eradication groups, respectively. Subjects followed more than 4 years were evaluated. Endoscopically, there were no differences in regression/progression of atrophy between the two groups (19). In contrast, histopathological analysis revealed significant regression of inflammation, atrophic gastritis and metaplasia in eradicated subjects. Individual analysis revealed that significant regression of atrophic gastritis in eradicated subjects was observed regardless of gender or age. In relation to intestinal metaplasia, significant regression in the eradicated group was observed in the overall analysis. These results suggest that *H. pylori* eradication induces regression of gastric pre-malignant conditions. However, whether or not mass eradication represents a cost-effective strategy to prevent gastric adenocarcinoma is still unknown.
Food can be supplemented with anti-

Helicobacter pylori substances to inhibit adhesion and growth of H. pylori. Professor Shigeru Kamiya (Kyorin University, Japan) addressed this novel issue. Many substances such as polyphenol, fucoidan, cocoa, lactoferrin and evening primrose have been reported to inhibit growth of H. pylori. A variety of food protein-derived melanoids inhibited urease-gastric mucin adhesion and Melanoidin I suppressed adhesion of H. pylori to gastric epithelial cells (20). These anti-H. pylori substances are useful for prevention of H. pylori colonization at primary infection and might be supplementary agents for H. pylori eradication therapy. However, probiotics such as Lactobacillus gasseri, L. salivarius, L. rhamnosus, L. acidophilus, Clostridium butyricum, Bacillus subtilis and Weissella confusa have been reported to inhibit the growth of H. pylori in vitro and in vivo. Production of organic acids by probiotic bacteria suppresses the growth of H. pylori and probiotic bacteria inhibit the adhesion of H. pylori to gastric epithelial cells (21). Because probiotics have the capability to regulate bowel functions, the use of probiotics with triple eradication therapy could decrease the occurrence of diarrhea caused by antibiotics. However, it is not clear whether probiotics are effective for eradication of H. pylori. Immunization with various virulence or colonization factors (urease, CagA, VacA, catalase, heat-shock protein) of H. pylori has been reported to prevent or decrease the colonization of H. pylori in animal studies. Clinical trials indicated that H. pylori vaccines (formalin-inactivated whole H. pylori cells, urease, CagA, VacA and NapA) with adjuvant LT-induced immune responses to H. pylori without serious side effects, indicate the safety of the vaccines. Prophylactic vaccination for children and therapeutic vaccination for the patients to whom eradication therapy was not successful are recommended and further clinical trials need to be performed.

Closing address

The symposium was closed with a summary by Professor Martin Blaser (New York University), in which he thanked both speakers and attendees. He outlined that the symposium focused on eight micro-organisms: six viruses, one bacteria and one worm, and that scientific progress was most evident, at least in part because these agents already have been identified as playing roles in cancer causation. Cellular, molecular and experimentally designed biological models have been applied to the basics of the identification of these agents, and their variation as well as their contributions and pathways to disease. However, there are still many unresolved questions and continuous research on infection, cancer and prevention must occur. Improvement and vaccine therapy and advances in whether or not micro-organisms might play important roles in other cancers are expected. The collateral benefit of such a symposium on the transmission of information among related areas as well as cross-collaboration between institutions cannot be over-emphasized. More importantly, the studies of the basics of microbial host interaction has benefits in understanding the pathogenesis process, developing new treatment modalities and the development of a new generation of investigators.

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