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Meeting Report

Sixth Sapporo Cancer Seminar

The Sixth Sapporo Cancer Seminar on Viruses, Immunodeficiency, and Human Cancer was organized jointly by T. Osato (Sapporo, Japan) and D. Purtilo (Omaha, NE). The meeting focused on three viruses: EBV,[1] HTLV-I (adult T-cell leukemia virus), and HIV, also known as HTLV-III or lymphadenopathy-associated virus. The biological similarities among the three viruses come in pairs. All three are lymphotropic in humans, but only the first two are transforming. EBV has a predilection for the B-lymphocyte, whereas the other two affect a specific subclass of T-cells. EBV and HTLV-I immortalize their lymphocyte targets and contribute to the genesis of certain human lymphomas or leukemias in a fairly straightforward, although somewhat indirect fashion. HIV is nontransforming and cytopathic. Lymphomas and Kaposi's sarcoma can arise in HIV-infected hosts, but they are attributed to the breakdown of immune surveillance against cells transformed by other viruses, rather than to a direct oncogenic effect.

The tumors that tend to arise in these and other immunodeficiencies, whether congenital or iatrogenic, are not representative of the major types of human cancer. They are nevertheless of very great interest because they reveal the existence of immunosurveillance mechanisms that prevent the outgrowth of virally transformed cells in immunocompetent hosts. Experimental tumor immunology has already taught us that virus-induced tumors are the best immunosurveillance targets. This is particularly true for the DNA tumor viruses. This is understandable, because their transforming sequences are essential parts of the viral genome. Ubiquitous viruses like EBV or polyoma that have imposed a strong selection pressure on their natural host species over long periods of time have brought about the fixation of multieffector but largely T-cell-dependent reactions, targeted against the direct or the indirect products of the early, transforming regions of the viral genome. The RNA tumor viruses do not have their own indigenous transforming genes. The directly transforming, acute, or class I retroviruses transduce cellular oncogenes that have been picked up by recombination. Such viruses play probably no significant role in natural tumor causation, but they are very useful as laboratory models. Nontransforming, chronically acting, or class II retroviruses may act by the somewhat more natural model of *cis*-insertion in the neighborhood of a cellular oncogene. Host surveillance may exist in these retroviral systems as well, but it is relatively handicapped, compared to the DNA tumor viruses, since it is usually focused on a helper virus-encoded protein that is easily dispensable, rather than on the transforming protein itself. In cases where the virus acts in a different way, *e.g.*, by expanding the target cell population at risk, as postulated for HTLV-I, antiviral immune responses may play a major role in modulating the likelihood of the disease.

Epstein-Barr Virus

EBV, the first genuine transforming virus indigenous to our own species, holds the record among all known transforming viruses in more than one respect. It is unparalleled in ubiquity and known age. The latter is somewhere in the range of 3 to 50 million years, the approximate age of our species and the time when the Old and New World primates separated. Its presence in the majority of all human populations reflects a highly successful transmission, in combination with an almost negligibly low disease risk. Its quiescence (latency) is no doubt an important part of its success. Its finely poised relationship with its experimentally best known host cell, the B-lymphocyte, is not far from a true symbiosis. The special nature of this relationship is reflected at several levels: the adaptation of the virus to a B-cell-specific surface moiety as its receptor; the action of the virus as a polyclonal B-cell activator; and the largely unexplored but proved existence of B-cell differentiation-dependent factors that govern the choice between the growth-transforming and the lytic virus-cell interaction. We know much less about the second major host, the epithelial cell, that can support the lytic multiplication of the virus. Also, while nasopharyngeal carcinoma is undoubtedly due to the uncontrolled proliferation of EBV-carrying epithelial cells, the nature of this virus-cell relationship and its role in the natural history of the tumor is as obscure as it was in the mid-1970s, at the time when the connection became firmly established.

There is also a well balanced relationship between the virus-transformed B-cell and the immune system of the host. Disease occurs only as a biological accident. There are four such accidents: infectious mononucleosis, EBV-carrying lymphoproliferative disease in immunodefectives, BL, and nasopharyngeal carcinoma.

A major part of the EBV-oriented talks was devoted to the lymphoproliferative disease in immunodefective hosts. This area was pioneered by Purtilo's discovery of the X-linked lymphoproliferative disease. On the basis of serendipitous clinical and pathological observations, Purtilo has suggested that X-linked lymphoproliferative disease was due to the unchecked proliferation of EBV-carrying cells in congenitally immunodefective children. His theory received strong support by the frequent detection of EBV genomes in the lymphoproliferative tissues. Purtilo has further suggested that the proliferations start as the polyclonal growth of EBV-carrying cells but may eventually progress to monoclonal lymphoma by a subsequent genetic change. Purtilo's general concept received strong support at the Sapporo meeting by the report of D. W. Hanto (St. Louis, MO), concerned with lymphoproliferative diseases in organ transplant recipients. He presented a first classification of these conditions on the basis of clinical and histopathological features, IgH rearrangement, light chain clonotype, EBNA staining, EBV DNA detection and cytogenetic analysis. One relatively young group of transplant recipients (mean age, 21 years) developed a mononucleosis-like illness after a relatively short latency. Initially, these lesions were polyclonal, but part of them progressed to monoclonality in the course of time. An older age group (mean, 47 years) developed localized, monoclonal tumor masses after a long latency period. Acyclovir therapy was effective in the first group, suggesting that continuous viral reinfection plays an important role in the pathogenesis of the disease. It had no effect on the second group.

The multieffector concept of EBV surveillance is in line with

[1] The abbreviations used are: EBV, Epstein-Barr virus; HTLV, human T-cell lymphotropic virus; HIV, human immunodeficiency virus; BL, Burkitt's lymphoma; EBNA, Epstein-Barr nuclear antigen; AT, ataxia telangiectasia; LCL, lymphoblastoid cell lines; LTR, long terminal repeat; ATL, adult T-cell leukemia; IL-2, interleukin 2.

the earlier finding that T-cell-mediated immunity against EBV-transformed cells may become undetectable in patients with impaired T-cell responses, *e.g.*, in Hodgkins' disease or non-Hodgkins' lymphomas, but there is no evidence of any EBV-carrying lymphoproliferative disease. Similarly impaired anti-B-EBV (EBV-transformed B-cell) responses were found by Katsuki and Hinuma (Kyoto, Japan) in HTLV-I-infected patients and by Osato and colleagues (Sapporo, Japan) in AT and Wiscott-Aldrich syndrome. No EBV-carrying lymphoproliferative lesions occurred in the HTLV-I carriers but they were occasionally present in both AT and Wiscott-Aldrich syndrome patients. It would obviously be important to test all lymphoproliferative lesions in immunodefective persons for the presence of EBV genomes, in order to establish the regularity of the viral involvement.

Chromosomal instability is another characteristic feature of AT, in addition to immunodeficiency. Osato's group has detected frequent chromosomal changes in LCL derived from AT patients, in contrast to normal controls. This was paralleled by an increase in agarose clonability and nude mouse tumorigenicity.

Zeng Yi (Beijing, People's Republic of China) and his collaborators have previously explored the prospective value of EBV serology in nasopharyngeal carcinoma. They found that IgA anti-early antigen and IgA anti-viral capsid antigen antibodies, originally detected by W. and G. Henle, were particularly useful for early diagnosis. Their cumulative data gave strong support to this conclusion and have also emphasized the clinical usefulness of the serological screening of high risk groups.

Several speakers dealt with the EBV-encoded proteins associated with growth transformation and with the lytic cycle, respectively. G. Klein (Stockholm, Sweden) described a new member of the nuclear antigen family designated as EBNA-5 (1). This protein is encoded by the *Bam*WY region of the viral genome. Its size varies between 42 and 90 kilodaltons in different monoclonal LCLs. Polyclonal populations of EBV-infected B-cells express a ladder of six or seven proteins, spaced at a distance of 6 kilodaltons, corresponding to different numbers of W repeats. Each monoclonal LCL expresses only one of them. BL lines do not express EBNA-5. Primary infection of the EBV-negative Ramos line does not induce EBNA-5, in spite of the good expression of the other four EBNAs. This is thus far the most striking phenotype-related differential expression of an EBV-encoded, transformation-associated protein. Similar but less pronounced differences have also been noted for some of the other proteins, however. The latent membrane protein and two other nuclear antigens, EBNA-3 and EBNA-4, are present in smaller quantities in BL than in LCL lines (2). The EBNA-1 and EBNA-2 coding region is occasionally deleted in BL, but not in LCL lines. Only EBNA-1 appears to be expressed at an equal level in BL and LCL lines.

Phenotypic differences between BL and LCL lines were also discussed by Y. Ishii (Sapporo, Japan) and G. Klein. Burkitt's lymphoma biopsies and early *in vitro* lines express a spectrum of monoclonal antibody-detected markers that are normally present on resting rather than activated B-cells. This is in line with the concept (3) that the BL cell can be regarded as a "suspended memory cell," *i.e.*, a B-cell that had expanded clonally under the impact of an earlier antigenic stimulus and was about to return to the resting stage upon the waning of the stimulus but has been prevented from reaching full quiescence (G_0) by the accidental intervention of the *myc*/Ig translocation.

The phenotype of the EBV-carrying (but not EBV-negative) BL lines tends to "drift" during prolonged *in vitro* cultivation and become more "LCL-like." Similar phenotypic switches can also be induced in vitro by converting EBV-negative BL lines into stable EBV-carrying sublines. Doi and Tatsumi described what may be a similar phenotypic switch in the first reported case of an *in vitro* EBV-transformed follicular lymphoma. Using clonal markers, they have clearly shown that the *in vitro* line represented the same clone as the original EBV-negative tumor but has partly switched its phenotype in the "LCL direction."

M. Nonoyama (Florida), G. Pearson (Washington, DC), and T. Sairenji (Worchester, MA) dealt with the antigens of the lytic EBV cycle. Nonoyama has identified 72 different mRNAs in virus-producing cells. Pearson and also Sairenji have identified new members of the early antigen complex. Nonoyama discussed the control mechanisms involved in the switch from the growth-transformed stage where all the lytic messages are switched off, to the productive viral cycle. He suggested that the *Bam*M region of the viral genome may play an important role in initiating the cycle.

Nonoyama has also confirmed the recent finding of Wang *et al.* (4) that the LMP (membrane protein)-encoding region of the viral genome could transform NIH-3T3 fibroblasts. He found a similar transforming activity with the *Bam*F fragment, when coupled to a retroviral LTR. While these experiments are of interest in showing the transforming potential of different subregions of the viral genome, similar experiments will be more directly relevant if performed on B-lymphocytes at different stages of differentiation. It has been widely documented that activated oncogenes can only transform cells at specific stages ("windows") of maturation. This "conditioned" tumorigenicity of activated oncogenes appears to be a general rule (5). The true B-cell-transforming potential of different EBV subgenomic fragments, alone or in combination with constitutively activated cellular oncogenes (*e.g.*, c-*myc*), remains to be assessed.

The tumorigenic phenotype can be antagonized by cellular genes that may be referred to as "tumor suppressor genes." A potentially relevant study was concerned with the role of the translocated *myc* gene in the genesis of Burkitt's lymphoma and the analogous animal tumors, mouse plasmacytoma, and rat immunocytoma, a key issue in the oncogene field. Oikawa *et al.* showed that the tumorigenicity of mouse plasmacytomas was suppressed when they were fused with fibroblasts, but not by hybridization with spleen cells. This is in line with earlier evidence. The Japanese workers have now also shown a parallelism between tumorigenicity and the expression of the translocated *myc* gene in their hybrid segregants.

Mizuno *et al.* (Sapporo, Japan) followed the work of the late Yohei Ito who had suggested that a correlation may exist between the occurrence of nasopharyngeal carcinoma and excessive consumption of phorbol-rich herbs. Mizuno has found a certain parallelism between the distribution of phorbol-containing plants and the high endemic African Burkitt's lymphoma region. He has also shown that phorbol esters promote the agarose clonability of EBV-transformed B-cells. The main weakness of this interesting hypothesis lies in the difficulty of proving or disproving its postulated role in the genesis of African BL.

Human T-Cell Lymphotropic Virus I

The sessions on the T-cell-tropic human viruses were started by a masterly presentation of the T-cell receptor genes by T. Mak (Toronto, Canada). The three T-cell receptor genes rearrange at different times during T-cell development. T-γ is the

first, T-β rearranges later, and T-α comes last. T-cell receptor gene rearrangement tests are useful for the distinction between polyclonal and monoclonal lesions. The monoclonal nature of chronic T-cell lymphocytosis, found by both T. Mak and Okabee (Sapporo, Japan), was an unexpected outcome of such studies.

Mak has also reviewed the T-cell lymphoma- and leukemia-associated translocations. In contrast to the regularity and uniformity of the *myc*/immunoglobulin juxtapositions in the B-cell-derived tumors already mentioned, T-cell leukemias show a great diversity of changes. Regular associations between specific translocations and distinct cellular subtypes are conspicuously absent. This does not mean that the translocations are necessarily epiphenomena. The opposite is suggested by the fact that several of the repeatedly registered reciprocal translocations involve the breakage of one chromosome in the neighborhood of a known or putative oncogene and another chromosome in the region of the T-cell receptor α or β gene. The fact that none of these affects more than a minority of the leukemias of a given morphological type is reminiscent of the correspondingly diverse oncogene activation by retroviral insertion in murine T-cell leukemia where known and putative oncogenes and preferential insertion sites have involved c-*myc*, c-*myb*, *pvt*-1, *pim*-1 Mlvi-1, and Mlvi-2. While a retroviral insertion in or near these genes could be registered in a substantial fraction of T-cell leukemias induced by a given virus, there was a similar lack of regularity as in the corresponding human T-leukemia-associated translocations.

Hinuma (Kyoto, Japan) reported that there were approximately 1 million HTLV-I carriers in Japan. In addition to Southern Japan and the Caribbean, a third endemic region has been discovered in Africa. The African and the Japanese virus isolates were virtually identical. Still another group with a high antibody incidence has been discovered on Hokkaido Island itself, among the Ainus. The age distribution was rather peculiar, with only 10% seropositives among the 10–19-year-olds but more than 45% in the age group above 60. This does not fit with the known modes of transmission via milk, blood, or semen. Hinuma raised the possibility that an initial state of relative tolerance may break down with age.

Hinuma's finding of frequent seropositivity among the Ainus received independent confirmation from the report of Iwanaga and Osato (Sapporo, Japan) showing that 10% of the antiviral antibody-positive ATL patients in Hokkaido were Ainus.

Three speakers dealt with the gene structure of the HTLV-I and -II viruses. Miwa (Tokyo, Japan) summarized his important work on the peptide deduced from the pX (*tat*) region of the viral genome. Antibodies against this peptide have permitted the identification of the pX protein and the study of its postulated *trans*-activating function on the viral LTR and possibly analogous regulatory regions in the cellular genome. A 21-base pair sequence target has been identified within the LTR. Yoshida (Tokyo, Japan) reported that the same sequence can provide enhancerless SV40 constructs with enhancer activity.

In an attempt to study the biological function of the pX protein, Miwa has introduced the *tat* gene, coupled to a zinc-sensitive Mt-1 promoter into a myoblast/teratoma cybrid. Expression of the gene inhibited myoblast differentiation. It would be very important to know whether the protein can also inhibit the maturation of T-cells.

Miwa has also reported 4 cases of adult T-cell leukemia that contained no detectable HTLV-I genomes. This is perhaps not so surprising as it would appear at first sight. A parallel may exist with the EBV-negative Burkitt's lymphomas. If the main role of the virus consists of stimulating the proliferation and expansion of the target cell population that is at risk to turn into a monoclonal malignancy by a subsequent and mechanistically unrelated genetic change, one would expect to find some virus-free leukemias. In further analogy with the EBV-negative BLs, they may be expected to occur all over the world, but at a much lower frequency than the virus-related cases in the high endemic regions. In Burkitt's lymphoma, the crucially important, virus-unrelated genetic change can be readily identified as the cytogenetically visible Ig/*myc* translocations. EBV-negative BLs carry the same translocations as their EBV-positive counterparts. They occur everywhere, but at an approximately 200-fold lower frequency than the high endemic tumors that carry EBV in 97% (but not 100%) of the cases. In the most commonly accepted BL scenario, EBV plays a role similar to what HTLV-I may play in ATL: expansion of the preneoplastic target cell population.

Another pioneer of HTLV-I, M. Yoshida (Tokyo, Japan), presented a more detailed HTLV-I/ATL scenario. He found that both the virally transformed T-cell lines and the HTLV-I-carrying leukemias were monoclonal, with a single random viral integration site. It is therefore unlikely that the virus acts by insertional activation of a cellular oncogene. The pX protein is localized in the cell nucleus. It can be regularly demonstrated in HTLV-I-carrying T-cell lines *in vitro*. Fresh leukemia cells are negative in Yoshida's experience. One positive case has been reported, following the use of the anticomplement immunofluorescence technique (6). The postulated role of the pX protein as the main transforming gene, believed to act by the *trans*-activation of cellular genes, needs to be reconciled with the nearly consistent absence of the protein from the leukemia cells.

In Yoshida's scenario, the primary infection induces the appearance of the IL-2 receptor on a subpopulation of T-cells. This leads to IL-2-dependent expansion of the infected cells. Highly antigenic cells keep being rejected. Antigen-negative variants survive preferentially. ATL arises when one of them escapes host control by a second (unknown) event.

Hatanaka (Kyoto, Japan) found that the viral genome can also be expressed in nonlymphoid cells. Infected HeLa cells form syncytia. Transfected pX could also be expressed in a natural killer cell line, but without inducing the IL-2 receptor. Yoshida's and Hatanaka's findings agreed in showing that while the induction of the IL-2 receptor in the appropriate T-cells could be a direct action of the pX protein, the inducibility of this receptor was dependent on the differentiation program of the cell.

Okochi (Fukuoka, Japan) discussed the transmission of the virus by blood transfusion. In four cases, seroconversion followed the transfusion of antibody-negative blood. This raises the important question of false negatives. Yoshida described his attempts to develop a vaccine and stressed the potential usefulness of the envelope protein as the inducer and target of neutralizing antibodies.

Yoshiki (Sapporo, Japan) succeeded in transforming rat T-cells with HTLV-I. They were immortalized and transplantable to newborn, but not adult syngeneic recipients. They may provide an interesting animal model for immunological and other biological studies.

Human T-Cell Lymphotropic Virus III (HIV)

The current global situation was reviewed by P. Ebbesen (Århus, Denmark). Extrapolation of the present trends suggests

that there may be 300,000 AIDS patients and between 3 and 10 million virally infected individuals by the year 1991. Most of the AIDS patients of 1991 are already infected today. The probability that AIDS will develop in a seropositive person is still relatively unclear, due to wide variations between different statistics. In a Danish report, the frequency was about 10% over a 3-year period, whereas a Manhattan study showed an even more alarming 30%. The heterosexual spread in Haiti and in Africa, including female to male transmission, is a cause for particular concern.

D. Volsky (Omaha, NE) reported the isolation of multiple viral genotypes from a single patient, suggesting infection by multiple viruses. The prevalent type can change in the course of the disease. There was also a high variability among viral isolates between different patients while established carrier lines showed a high degree of viral stability *in vitro*. Similar findings have been made previously on the herpes viruses, suggesting that *in vitro* persistence favors stability, while horizontal transmission between different individuals promotes genetic variation.

In order to approach the role of HTLV-III-induced *trans*-activation in latency and cytotoxicity, Volsky examined the expression of surface proteins in T4-positive cells. Several proteins were present in a reduced quantity, but without any detectable decrease in the corresponding message, suggesting that the *tat* gene may also act at the posttranscriptional level.

The AIDS situation in Japan was surveyed by Abe (Tokyo, Japan). A high incidence of seropositives was found in a group of hemophiliacs who received a certain preparation from the United States. This was also seen in China, as reported by Zen-Yi. Kurimura (Yonago, Japan) confirmed that the virus was occasionally transmitted from the female to the male. Abrams (San Francisco, CA) showed that the demographic, virological, and immunological behavior of PGL was fully comparable to the corresponding features of AIDS. He identified several symptoms in seropositives that were associated with a particularly high risk of AIDS development, with oral candidiasis and hairy leukoplakia as the leading representatives. In a broad clinical study on pediatric AIDS patients, Rubinstein showed that i.v. immunoglobulin can delay the occurrence of opportunistic infections.

The Sixth Sapporo Cancer Seminar clearly showed the usefulness of discussing the human lymphotropic viruses jointly at comparative meetings of this type. Whether DNA or RNA, transforming or lytic, T- or B-cell tropic, the virus-cell and cell-host relationships show a sufficient number of similarities and differences to make the comparison interesting and mutually rewarding.

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