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Meeting Report

First International Symposium on Epstein-Barr Virus and Associated Malignant Diseases[1]

Background and Objectives

Since the discovery approximately 20 years ago of EBV[2] by M. A. Epstein of the Medical School, University of Bristol, England, an immense body of information has been accumulated on this important member of the herpesvirus family. EBV was shown by G. Henle and W. Henle, Children's Hospital, Philadelphia, PA, to be the causative agent of IM. Subsequent studies by many investigators indicated an etiological association of EBV with BL and NPC. The postulated etiological role of EBV in BL and NPC was the result of extensive epidemiological studies. In addition, the EBV genome was detected in tumor cells and/or tissues by investigators in the United States, the United Kingdom, West Germany, France, Sweden, Hong Kong, Singapore, the Peoples Republic of China, and countries in Africa and the Middle East. For the last few years, EBV has also been shown to play a major role in the etiology of B-cell lymphoma in the immunodeficient populations of kidney and heart transplant patients. More recent findings suggest that EBV is also associated with the BL-like lymphomas which occur at high frequency in individuals with acquired immunodeficiency syndrome. Thus, this virus has the characteristics of a *bona fide* human cancer virus and continues to attract worldwide interest.

In 1975, an international workshop was sponsored by the National Cancer Institute to address the problem of EBV production, thus facilitating more basic research. Following this workshop, progress in both clinical and basic research on EBV has been presented in international meetings on herpesviruses at which advances, primarily in basic research, were discussed in conjunction with research on other animal and human herpesviruses, *e.g.*, herpes simplex, cytomegalovirus, and varicella zoster virus; and secondly at international symposia on NPC or BL where emphasis was on clinical applications of EBV research.

Because of the rapid advances in both basic and applied research on this internationally important virus, EBV investigators in the United States and abroad initiated a separate meeting on EBV in 1984 to review and disseminate new basic and clinical information. This meeting was expected to attract clinicians, epidemiologists, immunovirologists, geneticists, and scientists involved in environmental carcinogenesis. Specifically, the aims of this long overdue symposium on EBV and associated malignant diseases were: (*a*) to define more clearly the various disease manifestations associated with EBV infection throughout the world, including epidemiological, immunopathological, and genetic characteristics; (*b*) to discuss recent advances in basic research on EBV at the molecular level, including progress in mapping of the EBV genome and the identification of genes and gene products responsible for transformation, the purification and characterization of EBV proteins and the development of a common nomenclature for describing these proteins, and the development of new assays for monitoring both humoral and cellular immunity to EBV-infected and transformed cells; (*c*) to discuss new advances toward the prevention and treatment of EBV-associated cancers, including the development of a subunit vaccine against this virus and its potential application to human populations; (*d*) to stimulate active communication and interactions internationally between clinical and laboratory personnel in order to apply new laboratory findings more rapidly to a clinical setting; and (*e*) to establish among international scientists a forum for an active exchange of information and materials.

In addition, since specific chromosomal translocations have been found in BL as well as in other human cancers, the organizers and advisers thought it appropriate and timely to include the participation of investigators working with oncogenes in order to stimulate the exchange of information, materials, and active collaborations between investigators from different disciplines. The agenda included overviews in specialized areas by leading authorities in their respective fields, followed by the presentation of original investigations.

Session 1: Clinical Aspects of EBV-associated Diseases

The spectrum of clinical disease induced by EBV is greater than previously appreciated. In addition to the X-linked recessive lymphoproliferative syndrome and acute IM, attention was devoted to relapsing, recurrent, and chronic IM and to common variable hypogammaglobulinemia, with a thorough discussion of the clinical and laboratory features of the unusual sequelae of EBV infection. The participants noted the possible implications of the isolation of nontransforming as well as transforming strains of EBV from patients with varying clinical presentations.

Discussions of EBV-associated cancers focused primarily on NPC, with particular attention to a variety of approaches to the study of the genetic aspects of the disease, and BL, which

[1] The first International Symposium on Epstein-Barr Virus and Associated Malignant Diseases was held at Loutraki, Greece, on September 24 to 28, 1984. It was coorganized by S. D. Kottaridis, Hellenic Anticancer Institute, Athens, Greece; D. V. Ablashi, P. H. Levine, and T. Papas, National Cancer Institute, Bethesda, MD; and Gary Pearson, School of Medicine, Georgetown University, Washington, DC. The symposium was attended by approximately 90 investigators, representing 16 countries.

The meeting was sponsored through a travel grant by the National Cancer Institute and through funds provided by the Ministry of Health, Greece, the Greek Tourist Bureau, the Hellenic Anticancer Institute, and donations from many private biomedical companies from the United States and Europe.

The second international symposium, which will be held in the United States, is planned for October 1986.

The Proceedings of the International Symposium on EBV and Associated Malignant Diseases will be published as the first volume of *Developments in Medical Virology* by Martinus Nijhoff Publishing Co., Hingham, MA. It is anticipated that this publication will be published in 1985. This publication will serve as a reference text for the medical libraries and for investigators either already engaged in EBV research or intending to pursue research in the EBV field.

[2] The abbreviations used are: EBV, Epstein-Barr virus; BL, Burkitt's lymphoma; NPC, nasopharyngeal carcinoma; IM, infectious mononucleosis; EBNA, Epstein-Barr nuclear antigen; ELISA, enzyme-linked immunosorbent assay; ADCC, antibody-dependent cellular cytotoxicity; NK, natural killer; ACV, acyclovir; LYDMA, lymphocyte-detected membrane antigen.

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appears to be declining in incidence in sub-Saharan Africa but is increasing among young white males in the United States. Regarding the former, multidisciplinary approaches to the study of genetic predisposition to NPC are now in progress, and practical means of providing support facilities in high-incidence areas were given serious discussion. Reports of EBV association with salivary gland cancer and B-cell lymphoma in transplant patients suggested the need to broaden consideration of tumors possibly induced by EBV. In several reports, the possible interrelationship between EBV infection and newly identified retroviruses, such as human T-cell leukemia virus III/LAV, raised the potential importance of coinfection in patterns of disease.

The role of EBV serology in the early detection and monitoring of NPC was emphasized in several reports; the clinical value of IgA anti-viral capsid antigen, IgG anti-early antigen, and ADCC has now been confirmed. Controversy continues with regard to the relationship of EBV to the more differentiated forms of NPC. Immunoperoxidase assays to subclassify NPC further may be of value in this area.

Two new major research approaches discussed were (*a*) studies of the reactivity of the immune system during disease development by use of monoclonal antibodies and (*b*) correlation of immunovirological data with the disease process. In addition, single case histories of interest and special diagnostic procedures were presented. The fossa of Rosenmüller, a depression behind the pharyngeal opening of the eustachian tube along the lateral wall of the nasopharynx, was considered to be an important area for EBV investigation because of the initial histopathological changes observed in normal cells that result in NPC. A significant contribution to our knowledge of EBV-induced disease came from immunological findings showing that the type of disease which develops was highly dependent on the activity of the virus, its specific tissue tropism, and the defense system of the host at the time of infection or a change in host immunological status during latent infection.

Session 2

Molecular Biology of EBV. In this session on the molecular aspects of EBV, there was considerable new information presented since the preceding NPC/EBV International Symposium of 1982. Some of the new information indicated that integration of the total EBV genome occurs resulting in a deletion of host cell sequences and duplication of such sequences at the host-viral junction. However, the integration does not appear to be site specific, nor was there homology to known oncogenes at the junction of viral and host sequences. In addition, integration does not occur near the B-*lym* integration site. The *EBNA-1* gene, encoded in part by the IR3 region of the genome, contains a large number of repeats which vary in number and encodes for a gene product of variable molecular weight (M_r 68,000 to 85,000). A second EBNA protein (EBNA-2) is encoded in the IR1U2 region and has a molecular weight of 82,000. Since the EBNA protein arises from the 3′ end of a gene for which there is a long leader sequence, it is not yet clear whether EBNA-1 itself is required for the *trans*-acting function. Another finding demonstrated that the *BAM*HI K-fragment encodes for EBNA-1 and the *BAM*HI H-fragment produces EBNA-2. The detailed structure of the message arising from the IR1/U2 region of the EBV genome (*Bam*HI VXH) indicates that it contains 3 exons. It was proposed that an immediate-early gene may determine the fate of viral infection. Nucleotide sequencing is another approach for looking at the functional mapping of the EBV genome, particularly with regard to transformation *versus* lytic replication, and perhaps even promotion. It is expected that, after sequencing the entire EBV genome of the B95-8 EBV strain, the transcriptional program for EBV will fall into more than 3 classes. In fact, in the case of herpes simplex virus, subclasses of the 3 broad categories of messages have already been defined. A M_r 138,000 protein was detected in NPC tumors, and data were presented which indicated that antisera to this functional protein might be useful for clinical diagnostic purposes. Secondly, advances in nucleic acid hybridization technology indicated that it might be possible to use whole-organ sections for *in situ* hybridization and localization of the EBV genome in epithelial cells. A cyclic AMP-independent protein kinase (M_r 10,000) was identified which copurifies with nucleocapsids of purified EBV. It was shown that glycosylation was important not only for binding of virus but also for egress of virus from infected cells. Additional data on DNase antibody titers in the early course of NPC provided further evidence for the potential importance of this assay in management of patients with this disease.

Mechanism of EBV Transformation and Retroviral Oncogenes. This session reviewed the current concepts of oncogenes, facilitating understanding of their role in solid tumors, and the involvement of the c-*myc* gene in EBV-related BL. Detection of H-, N-, and K-*ras* genes in various tumor tissues indicates the importance of activated *ras* genes which have a point mutation in their coding region. c-*myc* activation at the site of 1 of the 3 immunoglobulin loci in BL appeared to be a regular feature in BL. A 3q+ chromosome marker with an involvement of band q25, either a duplication of the region q25-q27 or insertion of an unidentified segment at band q25, was reported in 2 EBV-carrying NPC tumors in 1983. Besides the above observations, nothing is yet known about the possibility of specific chromosome translocations and the role of transforming genes in NPC. However, it was considered important to probe NPC tissues for oncogene rearrangement and transcription and for the presence of transfection-detectable oncogenes. It was pointed out that all cells within the same tumor contain the same transforming gene whereas normal cells from the same patient did not. This suggests that activation of the transforming gene may be intimately connected with the processes leading to malignancy. Thus, exploration of the interaction between EBV, a highly transforming virus, and oncogenes was considered important for understanding EBV-implicated neoplasia. The data describing new chromosomal translocations (1:5) in North American BL suggested the involvement of another oncogene (c-*fgl*) in addition to c-*myc*.

The transformation and chromosomal translocation aspects in BL and NPC were extensively reviewed. There appear to be 3 regions of the EBV viral genome which are transcribed in transformed cells, LT-1, LT-2, and LT-3. LT-1 codes for the nuclear antigen now designated as EBNA-2 and which may be responsible for polyclonal B-cell activation; LT-2 codes for EBNA-1 and may be necessary for maintenance of transformation; and LT-3 may code for the membrane antigen LYDMA, the target of host immune surveillance. BL cells apparently have one and possibly 2 other activated oncogenes that participate in transformation.

The majority of the investigators thought that new terminology, *i.e.*, oncogenic transformation *versus* transformation or onco-

genic *versus* morphological transformation, would be confusing and would not add anything significant. Moreover, new terms, like growth transformation, should be avoided. Transformation of human lymphocytes obtained by transfection of purified EBV-DNA in the presence of the UV-inactivated virion of P3HR-1 EBV was interesting and may have future implications in EBV research.

The detection of EBNA and transforming EBV in human megakaryocytes suggested that the host range for EBV may be broader than had been anticipated. Thus, future investigations should focus on (*a*) immortalization of the cell type(s) encoded by the EBV genome and (*b*) the involvement and function of oncogenes in EBV-induced lymphoma cells and the epithelial cells of NPC. The area of cocarcinogenesis was considered important in understanding how certain factors, such as tumor promotors from plants and hormones (*i.e.*, hydrocortisone), interact with EBV to cause disease.

Session 3: EBV Proteins

This session dealt with identification and characterization of monoclonal antibodies that could be useful in the study of EBV proteins and their implications in the clinical setting and for vaccine development. A number of different EBV antigen complexes from EBV-infected or transformed cells were discussed. A fragment of EBNA synthesized in bacteria was used to detect EBNA antibody and to prepare polyclonal and monoclonal antibodies to EBNA. Such an antibody could be important in detection of EBNA in various EBV cancers. Secondly, the EBNA fragment (2.2-kilobase SmaI) was more sensitive in the ELISA assay than in the routine EBNA assay. In another approach, antibodies against a synthetic polypeptide identified EBNA in an ELISA test. Immunoblotting with the rabbit and human peptide-specific antibodies identified polypeptides that varied in size between M_r 70,000 and M_r 92,000 in different EBV-carrying cells. Two forms of major (M_r 72,000) polypeptides of EBNA from Raji cells after purification were present in the transformed and lytic states of the cells. In addition to EBNA, a cellular protein (M_r 62,000) was identified by immunoblotting with human sera. The polypeptide was present in EBV genome-positive and -negative cells. Further analysis revealed that it shared antigenic determinants with the M_r 72,000 EBNA protein. In another presentation, microinjection of subgenomic DNA fragments into human epithelial cells, as well as mononuclear cells, identified EBV-specific early antigen expression associated with charon fragments (EB-2 and EBj-7) and *Bam*HI fragments.

It was interesting to note that EBNA and other antigens present in a variety of EBV-transformed cell lines (M_r 110,000 to 115,000 and M_r 92,000) but absent in EBV genome-negative cells had also been detected in sera from patients with rheumatoid arthritis in the immunoblot assay. It appeared that the M_r 92,000 antigen was distinct from the previously reported EBV-related antigens. Other technological advances in EBV monoclonal antibody research included *in vitro* production of human monoclonal antibodies after transformation of peripheral lymphocytes from immune donors.

Session 4: Cellular Immunology

This session reviewed the cellular immune responses which might contribute to the control of EBV infection. These included ADCC, NK cell activity, and EBV-specific IgA-blocking factor in NPC patients. The most important contribution of ADCC in the EBV immunology field was that high ADCC reactivity was associated with improved prognosis in NPC. NK cells, with their broad nonspecific lysis, have also been shown to lyse EBV-positive cells to some extent, following induction of the EBV cycle. The determinants involved in ADCC and NK reactions are distinct, and NK cell activity appeared to be mediated by an extracellular NK factor. It is not known whether the activated T-cells, which have been used to describe the blast response-associated lytic population, represent non-NK cells or NK precursors activated by lymphokines. The implication of LYDMA or a previously unrecognized antigen, EBV-specific cytotoxic T-cells, and the development of the T-cell regression assay were discussed. It also appeared that reduced T-cell responses were recorded in certain patients with NPC or rheumatoid arthritis. The finding that EBV-specific and both OKT8-positive and OKT4-positive cytotoxic T-cells were restricted by Class I and Class II HLA antigens was also presented. T-cell responses to EBV infection (IM) were extensively reviewed, indicating that cytotoxic HLA-restricted memory cells, specific for LYDMA, persist at high levels and may play an important surveillance role *in vivo*. Thus, LYDMA may represent the first line of defense controlling the proliferation of EBV-transformed cells. Besides defining the recognition of viral antigens by the T-cell system, it was mentioned that virus load *in vivo* is not necessarily reflected by the level of antibody titers.

Humoral and cellular evidence for immunosuppression in EBV-associated disorders suggested various mechanisms by which EBV may interact with the immunoregulatory system to trigger immunosuppression. Immune complexes were detected in patients with EBV-associated disorders, but their specific role in immunosuppression is unclear. The *in vitro* immunogenicity of human lymphoid tumor cells, using the mixed-leukocyte reaction, revealed reduced reactivity in tumor cell lines, which may be due to release of a soluble factor ($M_r > 20,000$) capable of inhibiting both mixed-leukocyte reaction and the lymphocyte responses to both PHA and EBV antigens. The data on depletion of monocytes indicated a marked increase in immunosuppression, and irradiation of monocytes reduced T-cell suppresion to the level observed in the absence of monocyte depletion. These data suggest that monocytes may play a counterinhibitory role in immunosuppression. Another presentation concluded that lymphoid cells detected in NPC tumors are indicative of an active immune response *in situ* involving T- and NK cells. It was shown that isoprinosine, an immunopotentiating agent, enhanced lymphocyte response to the EBV antigens and secondly that isoprinosine abrogated the inhibitory activity of the blocking factor found in the sera of IM patients. The concluding remarks in the Cellular Immunology session indicated that considerable progress had been made in the last few years on the anti-EBV cellular effector mechanisms. The precise role that various structural and non-structural antigens of EBV system may play in EBV infection and their interactions with the host immune system remain to be defined. Moreover, little is yet known about immunoregulation in EBV infections. Such investigations are needed for better understanding of EBV disease and care of patients.

Session 5: Control of EBV Infections

This last session dealt with overviews on treatment of NPC, clinical and pathological features in the treatment of BL, a per-

spective on the treatment of EBV infections, and prevention of EBV-associated malignant diseases. It was recommended that the current stage classifications of NPC be improved to evaluate the effectiveness of different techniques used in treatment; otherwise, comparisons between treatment centers will not be meaningful. There continues to be controversy regarding classification as well as the goal of chemotherapy in controlling primary tumor and distant metastases. In some centers, bone metastases respond very poorly to the chemotherapy now available; other centers prefer chemotherapy to radiation therapy, which appears to offer only partial success, for patients with Stage II and IV disease. Although the value of chemotherapy with commonly recognized chemotherapeutic agents or with investigative drugs seems to be limited, the new treatment for NPC included use of α-interferon as a radiosensitizer. In another combination regimen using cyclophosphamide, methotrexate, vincristine, and doxorubicin, 10 patients of 54 treated prior to radiation achieved a complete remission, while 24 achieved partial remission. ACV in NPC patients showed no effect on the progression of tumor size but did have an effect on EBV shedding and regression of polyclonal lymphoproliferative disorders in allograft recipients. The antiviral treatment of EBV infections mainly concentrated on ACV. Whether application of ACV earlier in the course of disease would improve efficacy was considered worth investigating. The data presented suggested that none of the antiviral drugs that primarily inhibit viral DNA replication had any effect on the maintenance of EBV episomes in latently infected cells or the initiation and maintenance of EBV transformation of B-lymphocytes. Some newer experimental drugs exhibit remarkable persistent effects that may eventually be useful in prolonged infection states characteristic of EBV.

Although the incidence and clinical features of Burkitt's lymphoma differ in endemic areas compared to the rest of the world, there are no major differences with regard to the initial response to chemotherapy. There is a striking contrast, however, in the outcome for patients who relapse. Few patients who relapse in the United States, for example, survive. In Uganda, however, one-half of all long-term survivors have relapsed at least once and as often as 6 times before achieving prolonged disease-free survival (4 to 10 years). The possibility that this is a consequence of a more powerful host antitumor response, by virtue of the expression of viral membrane antigens in EBV-associated Burkitt's tumors, should be entertained. Transfer factor with specific anti-EBV activity, which appeared to significantly increase the disease-free intervals in African patients with BL, seems to merit further investigation. It will be of considerable interest to determine to which pattern the North African EBV-associated BLs belong. A common feature of all BLs occurring within as well as outside of Africa was the possession of characteristic chromosomal translocations which involve the c-*myc* gene and immunoglobulin genes. It was stated that immunological differences may exist in BL from endemic and nonendemic areas. Such differences are not well defined. The possible development of an EBV vaccine to control the infections which lead to malignant disease (BL, NPC) was extensively reviewed and discussed. The vaccine proposed was an EBV subunit vaccine composed of EBV membrane antigen glycoproteins. The nature of these glycoprotein molecules, the radioimmunoassay for its testing, immunoabsorbent methods for its purification, procedures to enhance its immunogenicity, and the highly sensitive ELISA for estimating specific antibody were presented. The data on its experimental trials in cottontop marmosets, which are known to develop lymphoma upon EBV inoculation, were discussed, as well as the logistic problems regarding its mass production, and the identity of high-risk populations for testing such a vaccine remained to be worked out.

Concluding Remarks

Scientifically, the First International Symposium on EBV and Associated Malignant Diseases was successful because it brought together investigators from basic and clinical settings from all over the world to review their findings; to share their problems; to learn the state-of-the-art technology in treatment, control, and prevention of EBV-associated diseases and cancers; and to establish or renew research collaborations. Moreover, it opened doors for new collaborations in the area of oncogenes.

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